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EDITORIAL

Acta Pædiatrica Changes its Name

When I. Jundell in 1921 started this pediatric journal and coined the name *Acta Pædiatrica* there were no other periodicals with this name and therefore no risk of confusion with other journals. The title Acta Pædiatrica has apparently been very attractive since quite a number of pediatric journals which have appeared later on have adopted the same name as a rule with an addendum indicating the country of origin. Sometimes this name of origin of the journal has been omitted in references thus causing a risk of confusion with our Acta Pædiatrica. In order to avoid this possibility of error we started to use the same principles as *Index Medicus* in its references, giving in brackets the name of city from where Acta Pædiatrica is distributed (*Acta Pædiatrica (Stockholm)*). This, however removed somewhat of the Scandinavian character of the journal and made it look like a purely Swedish pediatric periodical. Following a proposal by colleagues in other Scandinavian countries, the Board of Acta Pædiatrica therefore at last decided to add *Scandinavica* to the old name thus following the pattern of a number of other Scandinavian medical journals. Our pediatric periodical has therefore from this issue on got its name changed to

Acta Pædiatrica Scandinavica

This change of name does not imply any change of the editorial policy of the journal, which remains the same. The

increase of the subscription price to Swedish kronor 75 has nothing to do with the change of name but is solely due to the heavily increased costs of printing and paper *Acta Paediatrica Scandinavica* remains a product of a non profitable enterprise.

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From the Departments of Clinical Chemistry and Paediatrics, University Hospital, Uppsala, Sweden

The Concentration of Glucose in Whole Blood, Plasma, and Erythrocytes during the First Week of Life Determined by Different Methods and Evaluation of the Reliability of the Methods

by MAGNUS HJELM and STIG SJÖLIN

Low concentration of glucose during the first week of life has often been reported, and has been regarded as more or less normal for the period [18]. Recently however certain investigations have indicated that a low neonatal glucose level may be associated with pathological states such as neurological disturbances [3 9 16], hyperhaemolysis [13], and "subnormal function of the liver [2, 6 11 14].

If the observation is to be of any value, it is essential to know the true normal glucose concentration in blood, and especially its lower limit. Many attempts have indeed been made to determine this level, but the results have varied greatly [1 3 9 16, 18 20]. This is probably mainly due to differences in the methods used and also to the fact that the analyses have sometimes been made on whole blood and sometimes on plasma.

Methods based on the reducing power of glucose, *such as that of Hagedorn-Jensen*, give too high values owing to the presence in blood of reducing substances other than sugar The concentration of these substances differs in red blood cells and plasma. Hence the variations in the concentration of reducing substances in red cells and plasma and variations in the relative size of the two compartments directly influence the "glucose" value in whole blood.

The enzymatic glucose-oxidase methods were first thought to be extremely specific for glucose [12]. It has been shown, however that the method can be disturbed in different ways, and may give false low or false high values [12]. The few investigations on the glucose concentration during the neonatal period that have been made by the glucose–oxidase method do not seem to have taken these facts into account [1 3 9 16 20].

It is thus almost impossible to compare the results of different investigations and *to estimat their reliability* In fact, very little is known of the true level of glucose in blood and the individual variations during the first few days of life

The first purpose of this investigation was t determine simultaneously the con

A preliminary report of this investigation was given at the meeting of the Swedish Society of Clinical Chemistry on November 30th, 1961. The investigation was sponsored by the Swedish Medical Research Council (Project No. 064).

centration of glucose in whole blood, plasma, and erythrocytes from cord blood and capillary samples in infants during the first week of life, (1) by Hagedorn Jensen's method, and (2) by two modifications of the glucose-oxidase method. The second purpose was to determine the reliability of the methods used. It has been shown by Hjelm & de Verdier [12], that the quotient glucose-in-erythrocytes/glucose-in plasma, determined with the aid of C^{14} labeled glucose, is a reliable criterion of the accuracy of a glucose method. By using this criterion it was possible to evaluate the glucose values with all three methods. One of the methods seems to give reliable values for the concentration of glucose in blood during this period of life.

Material and Methods

Three different groups of infants were used. In the first group (A) a comparison was made between three different glucose methods. In the second group (B) the quotient glucose in-erythrocytes/glucose-in plasma was determined with C^{14} labeled glucose, and compared with the same quotient determined by one of the glucose methods. In the third group (C) the most reliable glucose method was used for the determination of normal glucose values during the first week of life.

Group A

Cord blood and capillary blood was investigated in a group of [illegible] fullterm, clinically healthy neonates. Cord blood samples were collected in heparinized test tubes containing NaF, immediately chilled, and stored at +4°C. The capillary samples were taken by heel puncture, collected in small heparinized test tubes, chilled, and deproteinized within twenty minutes. The first capillary sample was taken within one hour after birth and analysed simultaneously with the cord blood. Capillary samples were also collected four and eight hours after birth. During the day after delivery three blood samples were taken at 8 a.m., 1 p.m. and 4 p.m. just before a meal. The haematocrit values of the samples were determined (International Haematocrit Centrifuge, International Equipment Company, Boston, Mass., U.S.A.). One part of the blood was immediately taken for analysis; the rest of the sample was centrifuged and plasma and erythrocytes were separated. The haematocrit reading of the packed erythrocytes was determined in about 50% of the samples selected at random.

The *glucose concentration in whole blood, erythrocytes and plasma* was determined by the following three methods.

1. A method based on the reducing power of glucose and described by *Hagedorn-Jensen* [cf. 12].

2. A modification of the glucose-oxidase method, using 0.3 *M* perchloric acid as protein precipitating agent and with correction for the unspecific interfering oxidation of the chromogen, as described by Hjelm & de Verdier [12], the *PCA-GO method.*

3. A modification of the glucose-oxidase method, using 0.025 *M* NaOH and 10% $ZnSO_4 \cdot 7H_2O$ as protein precipitating agent and with correction for the unspecific oxidation of the chromogen, as described by Hjelm & de Verdier [12], the *Zn-GO method.*

Various concentrations of anhydrous glucose in saturated aqueous-solution of benzoic acid were used in order to check the first method and to standardize the second and third methods. The quotient glucose-in-erythrocytes/glucose-in-plasma was calculated for all samples. When determining the concentration of glucose in erythrocytes no correction was made for the small amount of trapped plasma ([illegible]%) in the packed erythrocytes.

Group B

In 5 cord blood samples and in 18 capillary samples from different newborn infants taken on different days during the first week

of III the quotient glucose-in-erythrocytes/glucose-in plasma (i.e. the quotient for the available glucose "water space" in erythrocytes/glucose "water space in plasma) was determined by using C^{14}-labeled glucose and the Zn–GO method.

Two hundreds ml of a solution containing about 0.15 µC of glucose uniformly labeled with C^{14} and with high specific activity (SFB.25, The Radiochemical Centre Amersham, England) was added t 1 ml of cord blood or capillary whole blood, collected in heparinized tubes and immediat ly chilled t bout 0°C. (The capillary samples were collected at 8 a.m., just before a meal.) The tubes were inverted several times for half a minute stored in ice-water for one minute, and centrifuged at 2500 g for 5 minutes t +4°C in an ordinary refrigerated centrifuge Plasma and erythrocytes were separated, and the trapped plasma in the packed red cells was determined. For counting the radioactivity 0.02 ml of plasma or of packed red cells was mixed with 100 µl of 2% glucose in water (t rinse the pipett and to obtain a smooth deposit) on an aluminium planchett and the mixture was evenly distributed over a circular area with a dia meter of 25 mm. The planchettes were counted in a gas-flow counter (Nuclear Chicago, Chicago Ill., U.S.A., Model D47 equipped with a Model M-5 semi-automatic changer). The activity was corrected for self absorbance on the planchett The quotient radioactivity in-erythrocytes/radioactivity-in-plasma was calculated. N correction was made for the minor amount of trapped plasma in the packed red cells (2–4%). In some cases venous blood from healthy blood donors was used to check the method and for comparison with previous investigations [11] In all samples the glucose concentration in erythrocytes and plasma was determined by the Zn–GO method, and the quotient glucose-in-erythrocytes/glucose-in-plasma was also calculated from these alues.

Group C

In a group of 16 full term, healthy infants the concentration of glucose in whole blood, plasma, and erythrocytes was determined by the Zn–GO method during the first week of life. The samples were collected as described for group A. The quotient glucose-in-erythrocytes glucose-in-plasma was calculated for comparison with the quotients obtained from the experiments with C^{14} labeled glucose

Results

1 *Comparison of the three methods*

The results of the comparative investigation on the glucose concentration in the three different media by the three methods are shown in Fig. 1 It is seen that the methods give considerably different mean erythrocyte glucose values; the highest values were obtained by the method of Hagedorn-Jensen and the lowest by the PCA–GO method. In plasma only a minor difference was observed between the glucose values determined by the Hagedorn-Jensen method on the one hand and the two other methods on the other The mean glucose concentrations of whole blood differed according to the method used, the Hagedorn-Jensen method gave the highest, and the PCA–GO method the lowest glucose values. The statistical analysis (see Appendix) shows that the differences in glucose concentration noted between the three methods are significant.

The mean values for the quotients glucose-in-erythrocytes/glucose-in plasma for each day each individual and each of the three methods are shown in Fig * The quotients obtained with the Hagedorn Jensen method are very high, and those found with the PCA–GO method extremelv low as can be expected from the very low values for glucose-in-erythrocytes with the PCA–GO method.

The quotients in capillary blood as

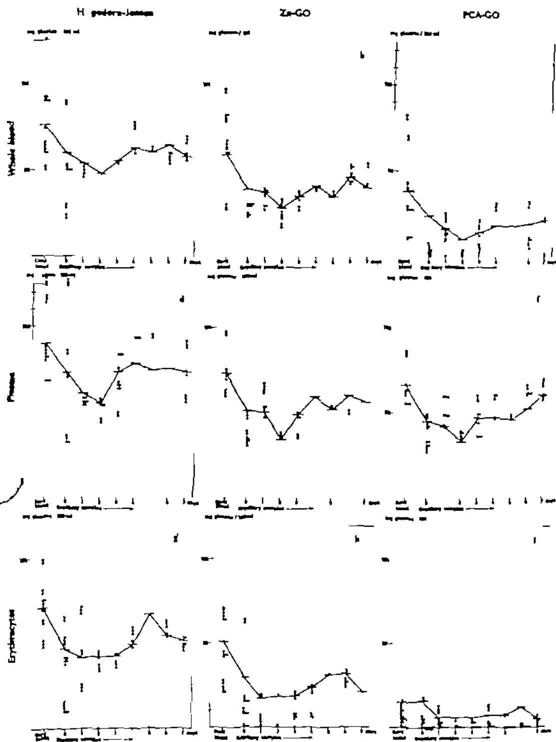


Fig. 1 Glucose concentration in whole blood, plasma and erythrocytes during the first week of life as determined by three different methods.

determined by the Zn–GO method are somewhat low during the first three days compared with values reported for adult blood [12, 15]

2 *Determination of the quotient glucose-in-erythrocytes/glucose in plasma with C^{14} labeled glucose during the first week of life*

In order to check the preliminary results the quotient for the distribution of glucose between erythrocytes and plasma was determined with C^{14} labeled glucose The result is presented in Table 1 The quotients determined with C^{14} labeled glucose and by the Zn–GO method tally closely The values for the quotient are lower in capillary blood than in cord blood and blood from healthy blood donors.

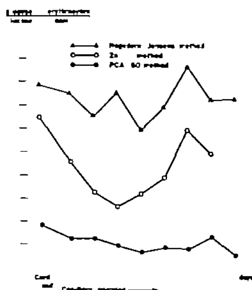


Fig. _. The quotients glucose-in-erythrocytes/glucose-in-plasma as determined by three different glucose methods.

3 *The concentration of glucose in whole blood, erythrocytes and plasma in healthy newborn infants*

The results are presented graphically in Fig 3–5 The individual curves and the mean values for the concentration of glucose in whole blood, erythrocytes, and plasma in 16 infants are shown. There is a significant drop ($P<0.001$) in the concentration of glucose from the cord blood to the first and subsequent capillary samples in all types of medium used. The lowest values are reached on the second and third days. A few values for whole blood are lower than 20 mg/100 ml, and a few for plasma lower than 30 mg/100 ml.

Fig. 6 shows the individual and mean values for the distribution of glucose between erythrocytes and plasma during

TABLE 1. *The quotient glucose-in-erythrocytes/glucose-in-plasma determined with C^{14} glucose ($Q_{C^{14}}$) and the Zn–GO method (Q_{ZnGO}).*

Cord blood		Capillary blood: Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day		Adults	
Q_{ZnGO}	$Q_{C^{14}}$	Q_{ZnGO}	$Q_{C^{14}}$	Q_{ZnGO}	$Q_{C^{14}}$	Q_{ZnGO}	$Q_{C^{14}}$	Q_{ZnGO}	$Q_{C^{14}}$	Q_{ZnGO}	$Q_{C^{14}}$	Q_{ZnGO}	$Q_{C^{14}}$	Q_{ZnGO}	$Q_{C^{14}}$	Q_{ZnGO}	$Q_{C^{14}}$
0.63	0.64	0.51	0.57	0.54	0.60	0.47	0.50	0.58	0.59	0.56	0.58	0.70	0.53	0.65	0.63	0.71	0.65
0.71	0.66	0.54	0.41	0.49	0.50	0.63	0.55	0.56	0.56					0.57	0.50	0.73	0.85
0.67	0.62	0.71	0.58	0.49	0.47	0.55	0.54									0.63	0.62
0.69	0.68	0.60	0.53													0.69	0.63
0.61	0.64	0.63	0.53														
Mean																	
0.67	0.65	0.66	0.53	0.51	0.53	0.53	0.56	0.57	0.58	0.56	0.58	0.70	0.53	0.61	0.57	0.70	0.70

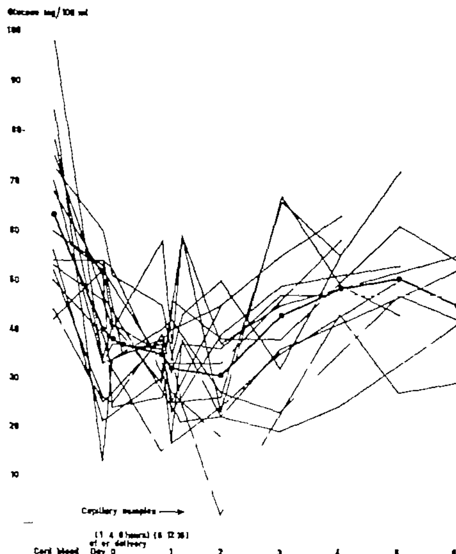


Fig. 3. Glucose concentrations in whole blood during the first week of life as determined by the Zn–GO method. ●—● Mean concentration.

the first week of life. The values for the mean quotient determined with C^{14} labeled glucose are also indicated. There is close agreement between the two mean curves, and there is a significant drop ($P<0.001$) from cord blood to the capillary quotient.

Discussion

Many investigations designed to establish the blood glucose during the neonatal period have been reported [1, 3, 9, 10, 18, 20]. The results have varied widely owing to differences in methods, and because both plasma and whole blood have been

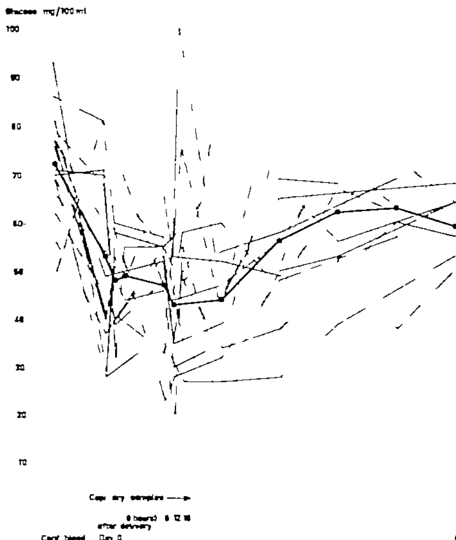


Fig. 4. Plasma glucose concentration during the first week of lif as determined by the Zn–GO method. ●—● Mean concentration.

used. In recent years a new method based on enzymatic determination of glucose has come into use and several modifications have been described (for references see [12]) Despite the marked specificity of the enzyme (glucose oxidase) for glucose a number of factors may interfere with the method giving false high and false low values, unless special precautions are taken [12]. This explains the extremely low glucose concentrations reported by Wolf during the first week of life [20]. And also in other investigations on the glucose concentration during the neonatal

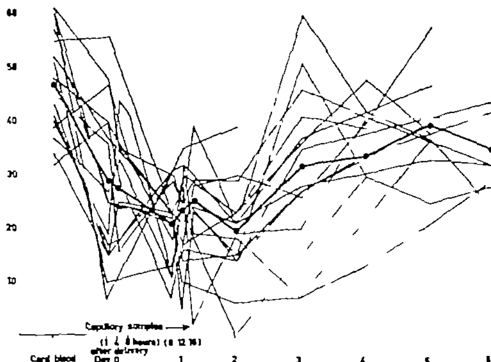


Fig. 5. Erythrocyte glucose concentration during the first week of life as determined by the Zn–GO method. ●—● Mean concentration.

period using the glucose-oxidase method insufficient attention has been paid to the interfering factors [12].

In the present investigation greatly varying glucose concentrations were recorded in each individual sample according to the method used. Comparison between different methods and media (whole blood, erythrocytes, plasma) illustrates the fallibility of blood-glucose determination, and largely explains the divergent reports It has proved possible to evaluate the reliability by calculating distribution quotients for glucose in erythrocytes and plasma as estimated by the various glucose methods, and to compare the quotients with quotients determined with glucose labeled with C^{14} [12 15]. The modified Zn–GO method alone gave true glucose values when the distribution of glucose between erythrocytes and plasma is used as a criterion of the reliability of the method. It has previously been shown that the Zn–GO method gives true blood glucose values in adults [12] and the same also seems to apply during the neonatal period. It is probably of importance in practice that the method gives true glucose values both in whole blood and plasma, because the determination of whole-blood glucose is simpler

The determination of the glucose con-

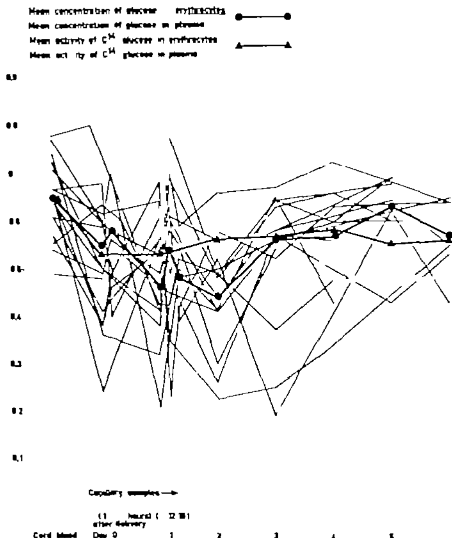


Fig. 6. Individual and mean quotients glucose-in erythrocytes/glucose-in-plasma as determined by the Zn–GO method and the mean quotient glucose-in erythrocytes/glucose-in-plasma as determined by the C^{14}-method.

centration by the Zn–GO method has yielded interesting results that may have physiological implications. The glucose level falls significantly during the first hour of life, and remains low during the neonatal period. This may be explained by e g a decreased glucose 6-phosphatase activity in the liver as has been described in newborn animals [4], with inhibition of liberation of glucose from the liver glucogen. The infant would then be unable alone to maintain the higher intrauterine glucose level, and the glucose level would drop soon after birth.

The distribution quotient glucose-in erythrocytes/glucose-in plasma is less in capillary blood than in cord blood. The fact that this change in the quotient was shown by both the C^{14} and Zn–GO methods is strong evidence of the reliability of the latter. The reason for the change in quotient is obscure. The finding, however, indicates that the water space for glucose in the erythrocytes is smaller in capillary blood of neonates than in cord blood. The increase in osmotic resistance of the erythrocytes after birth is probably a parallel phenomenon [17].

The lower limit of the glucose concentration is of clinical interest because low blood-glucose levels have been reported during the neonatal period in pathological states. The average plasma-glucose concentration as determined by the Zn–GO method was never less than 40 mg/100 ml, which is also the glucose-concentration in the water phase of erythrocytes. Only a few individual values were less than 30 mg/100 ml plasma (Fig. 4).

It has been suggested that the low glucose concentration might lead to decreased glycolysis in the erythrocytes with resulting insufficient ATP formation and increased haemolysis [13]. Hexokinase which catalyses the first limiting step in erythrocyte glycolysis, ought however to be saturated at this glucose concentration (30 mg/100 ml) since Michaelis-Menten's constant, K_m (glucose) for hexokinase in erythrocytes is much lower [8, 13], and the break-down of glucose ought therefore to be sufficient for the adequate formation of ATP.

Zinkham *et al.* [21] and Hollaender *et al.* [10] have reported the development of glutathione-instability on incubation of erythrocytes with phenylhydrazine *in vitro* when the initial glucose concentration of the incubation solution is less than 35 mg/100 ml. This glutathione instability appears to be due to cessation of the cellular metabolism before the end of the incubation period, the glucose in the solution probably having been used up. The finding is therefore probably of no significance *in vivo* and the glucose concentration as such is probably not responsible for the decreased life span characteristic of erythrocytes formed before birth and during the neonatal period [7].

Dipietro *et al.* [5] and Walker [19] found strong experimental evidence in animals that phosphorylation of glucose in the liver probably depends on two different kinases with highly different affinities for glucose. The one, hexokinase, has a very high affinity for the substrate, and is already present at birth, but probably only in small activities; it is not entirely specific for glucose but is also capable of phosphorylating other sugars. The other, glucokinase, is not formed until after birth, it has a low affinity for glucose but is specific for the substrate and has probably a greater activity than hexokinase. If the conditions are identical in man, the presence of only hexokinase may explain why the elimination of glucose injected intravenously is slower among infants during the first week of life than subsequently [2, 6]; this may also be the case with galactose [11, 14]. If some glucokinase were present at birth, the glucose concentration would probably be too low to saturate the enzyme and the initial phosphorylation of glucose would take place at a slower rate with secondary changes in liver metabolism as result.

Summary

The concentration of glucose in whole blood, plasma, and erythrocytes was determined by three different methods in 76 full-term, healthy newborn infants during the first week of life. The reliability of the methods was evaluated. With the most reliable of them, the Zn–GO method, the concentration of glucose in whole blood, plasma and erythrocytes was followed up during the first days of life in 16 full term, healthy infants. With the Zn-GO method the average plasma-glucose concentration was never less than 40 mg/100 ml. Only a few individual values were below 30 mg/100 ml plasma.

The importance of this glucose level for the breakdown of glucose in the erythrocytes and the phosphorylation of glucose in the liver is discussed.

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(M.H.). Department of Clinical Chemistry
(S. S.). Department of Paediatrics
Akademiska Sjukhuset
Uppsala
Sweden

Statistical Appendix

by Magnus Hjelm, Sig Sjölin, and Adam Taube[1]

In group A (cf. Material and Methods) the glucose concentration in whole blood, plasma, and erythrocytes was determined by three different methods for each infant on most days of the first week of life. The individual and mean glucose values are shown in Fig. 1 (p. 6). The mean glucose values obtained by the different methods apparently vary, but it is important to know whether the differences are systematic or only random. The following statistical treatment was therefore done.

For any of the types of blood samples (whole blood, plasma, or erythrocytes) analysed the determinations may be described as follows:

Method	Determinations	Mean
Zn–GO $= x$	$x_1, x_2, \ldots x_n$	$\bar{x} = 1/n\Sigma x$
PCA–GO $= y$	$y_1, y_2, \ldots y_n$	$\bar{y} = 1/n\Sigma y$
Hagedorn-Jensen $= z$	$z_1, z_2, \ldots z_n$	$\bar{z} = 1/n\Sigma$

Each determination may be considered as the sum of three components, the "true" value for the glucose concentration (μ_i) on day i, the systematic deviation from this value associated with the three methods used (α_{Zn-GO}, α_{PCA-GO} and α_{Hag-J}), and the random error of the determinations (ε_i^{Zn-GO}, ε_i^{PCA-GO} and ε_i^{Hag-J}) on day i. These factors determine the observed values (x_i, y_i and z_i) on day i as follows: Observed value = "true" value + systematic deviation + random error

$$x_i = \mu_i + \alpha_{Zn-GO} + \varepsilon_i^{Zn-GO}$$
$$y_i = \mu_i + \alpha_{PCA-GO} + \varepsilon_i^{PCA-GO}$$
$$z_i = \mu_i + \alpha_{Hag-J} + \varepsilon_i^{Hag-J}$$

[1] Institute of Statistics, Uppsala University, Uppsala.

The difference (d_i) on day i between the glucose values, e.g. with the Zn–GO and the PCA–GO method in whole blood, plasma, or erythrocytes for a certain infant may thus be expressed as

$$d_i = x_i - y_i = (\alpha_{Zn-GO} - \alpha_{PCA-GO}) + (\varepsilon_i^{Zn-GO} - \varepsilon_i^{PCA-GO})$$

but $(\varepsilon_i^{Zn-GO} - \varepsilon_i^{PCA-GO})$ has zero expectation, (i.e. the random errors will cancel each other). This means that the difference between the glucose concentrations as determined by the two methods is a measure of the difference between the systematic deviations from the true value with the same methods. Nothing can be said about the absolute values of the α-components as long as μ_i is not known.

For each child three differences have been calculated for whole blood, plasma, and erythrocytes, viz. (1) the difference between the glucose values determined with the Zn–GO and PCA–GO method, ( ) the difference between Hagedorn-Jensen's method and the mean of the sum of the values with the Zn–GO and PCA–GO method, and (3) the difference between Hagedorn-Jensen method and the Zn–GO method. (1) and (2) are results of "orthogonal" comparisons. The Zn–GO and PCA–GO methods are modifications of the same principle and Hagedorn-Jensen's method is a completely different method. The mean of the sum of the Zn–GO and PCA–GO methods is difficult to interprete biologically, however. (3) was therefore also calculated, but this difference is not a result of an orthogonal comparison.

For each child and difference it was confirmed that there was no significant variation among the capillary samples during the time of observation and that the difference was

TABLE 2.

	$\varepsilon_{Ba-GO} - \varepsilon_{PCA-GO}$			$\varepsilon_{Hag-J} - \varepsilon_{Ba-GO}$			$\varepsilon_{Hag-J} - \frac{1}{2}(\varepsilon_{Ba-GO} + \varepsilon_{PCA-GO})$			Expected frequencies of t values if the hypothesis is true
t Values	W	P	B	W	P	B	W	P	B	
Negative*	0	5	0	0	0	0	2	0	0	13
Positive, but not significant	10	19	15	9	15	4	16	17	9	12.35
Significant										
$0.05 > P > 0.01$	9	2	5	7	5	5	4	5	3	
$0.01 > P > 0.001$	3	0	6	7	4	6	3	2	13	0.65
$0.001 > P$	4	0	0	3	2	11	1	2	1	
No. of t values	26	26	26	26	26	26	26	26	26	26

W = whole blood, P = plasma, B = packed erythrocytes.
* None of the negative differences were significant.

equal in capillary and cord blood samples. Thus the difference, d is assumed to be independent of time and to be a measure of the difference between the systematic deviations of the methods. The three types of differences calculated above correspond to three null hypotheses, as follows.

$$\varepsilon_{Ba-GO} = \varepsilon_{PCA-GO},$$
$$\varepsilon_{Hag-J} = \tfrac{1}{2}(\varepsilon_{Ba-GO} + \varepsilon_{PCA-GO}),$$
$$\varepsilon_{Hag-J} = \varepsilon_{Ba-GO}.$$

These hypotheses can be tested with Student t test for *each child* and *each type of blood sample* in accordance with the formula,

$$t = \frac{\bar{d} - 0}{s_d \sqrt{n}} \qquad s_d = \pm \sqrt{\frac{\sum (d - \bar{d})^2}{n - 1}}$$
$$\bar{d} = \frac{1}{n} \sum d$$

where $\bar{d}$ = the mean value of the differences (d_1, d_2 ... d_n) for a certain child and type of difference, n = the number of differences for a certain child, and $n - 1$ = degrees of freedom.

If the systematic deviations from the true values in the methods show no difference it can be expected that among all the t-values for certain type of difference and a certain type of blood sample (e.g. $\varepsilon_{Ba-GO} - \varepsilon_{PCA-GO}$ in whole blood) 50% will be positive and 50% negative; and, further that 5% of the t-values will give at least probably significant values in either direction.

The result of the 26 t values in whole blood (W) plasma (P), and erythrocytes (E) of 26 neonates are shown in T ble 2. The distribution of t values differs from the distribution that would have been expected if no differences between the systematic devi ations had been present.

For a rough test of the over-all results the group with the highest incidence of negative t-values ($\varepsilon_{Ba-GO} - \varepsilon_{PCA-GO}$ in plasma) was chosen. The critical ratio for this group was calculated from the formula

$$\text{critical ratio} = \frac{f - np}{\sqrt{np(1-p)}} = \frac{5 - 13}{\sqrt{26 \cdot 0.5 \cdot 0.5}} = -3.14$$

where f = frequency of negative t values, np = expected numbers of negative t values, and $\sqrt{np(1-p)}$ = standard deviation of f

The calculated value exceeds the 2.58 found for a normal distribution, and is significant at the 99% level. The three null hypotheses can thus be rejected, and the same is clear if the distribution of significant t values is considered *Thus th differences in*

the mean glucose concentrations in Fig 1 seem to be true differences

The variation in the random error σ cannot be estimated in this material, since only single determinations were done The following values have been calculated on basis of duplicate determinations in earlier unpublished investigations using the formula $\sigma = \pm\sqrt{\sum d^2/2n}$ giving the following results

Method	Zn-GO		PCA-GO		Ha-dor Jen[illegible]	
Glucose concentration mg/100 ml	60	100	56	100	60	1[illegible]
σ_a, mg/100 ml	2.4	2.7	4.[illegible]	2.8	[illegible].4	[illegible]
N of duplicate determinations	4	24	28	28	23	5

Acknowledgements

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From the Section of Virology Department of Clinical Bacteriology Karolinska Sjukhuset and the Department of Virus Research, Karolinska Institutet, Stockholm, Sweden

Virologic Studies on Cytomegalic Inclusion Disease

by GUN CARLSTRÖM

The existence of characteristic intranuclear inclusions in cells of various viscera has been known since the beginning of this century [7 9] On the basis of histopathologi findings two different types of condition have been described, related to the extent of the pathological changes: (1) a focal process with the intranuclear inclusions localized in the salivary glands (2) generalized cytomegalic inclusion disease

Such changes are not uncommon. Thus specific inclusions confined to the salivary glands were found in more than 10% of the cases in a routine autopsy material of infants and young children [3]. These findings have contributed to the opinion that the presence of such localized inclusions are not necessarily associated with clinical disease.

The incidence of the generalized disseminated form has in some series of autopsies in infants been 1-2% [3]. In older children and adults it has been found much more infrequently [17].

A considerable diagnostic advance was made when, in 1952 Fetterman [4] demonstrated that infants with cytomegalic inclusion disease might excrete typical inclusion-bearing cells in the urine The finding of such cells thus allows the recognition of cases during life.

For many years the nature of these peculiar cellular changes was obscure. In the discussion on the aetiology protozoan parasites spirochetes and viruses were considered. Later experiments with transmission by filtrates in animals indicated the viral nature of the disease

The isolation in tissue culture of human intranuclear inclusion-producing virus strains was reported in 1956 by Smith [13] and by Rowe and his coworkers [11] and in 1957 by Weller and coworkers [15]. These strains, which appeared to be identical, were referred to as the cytomegaloviruses and considered representatives both of human salivary gland inclusions and generalized cytomegalic inclusion disease. Quite a few cytomegalovirus strains have since been isolated, mostly in the U.S.A. [12 16] There are also scattered reports from other parts of the world [6, 8].

The introduction of methods for isolation of cytomegalovirus and the development of serologic procedures have made it possible to investigate the incidence of infections to a greater extent than could earlier be done with existing pathological

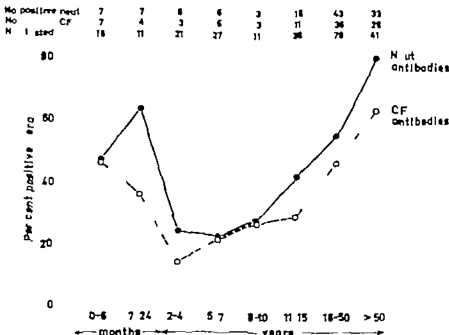


Fig 1. Distribution of neutralizing and CF antibodies in different age groups of 242 children and adults. Neutralizing antibodies tested at a final serum-dilution of 1/10 CF antibodies at final serum-dilution of 1/4

I olation of cytomegalovirus

T demonstrated incidence of anti es in children and adults of different ages indicated that cytomegalovirus infections often occurred in a Swedish population. Therefore efforts were made to isolate virus from infants treated in the paediatric department with the tentative clinical diagnosis of cytomegalic inclusion disease. The efforts soon yielded positive results, cytomegalovirus being recovered from a 4-month-old infant. Detailed clinical and cytological data on this case will be given elsewhere Human embryonic skin and muscle cell cultures inoculated with a mouth swab sample from this patient developed specific cytopathic changes in 10 days ("Ström strain") Small foci of enlarged round cells appeared with a slow progression in the fibroblast out growth. Except for deposition of granular material only minimal changes were observed for a couple of weeks. After 4 weeks the characteristic changes had progressed somewhat and passages were made with supernatant culture fluid containing whole cells, released by scraping or by treatment with trypsin. After 4 passages the incubation period was shortened to 2 days with complete degeneration occurring within 14 days. From this passage on, virus was present in the centrifuged supernatant fluid, and the titer of virus in fluids from the 5th passage was $10^{-4.4}/0.1$ ml.

In stained preparations, made at intervals throughout the passage series, the morphological changes were found similar to those previously described in cytomegalovirus infections [11], i.e. cells con-

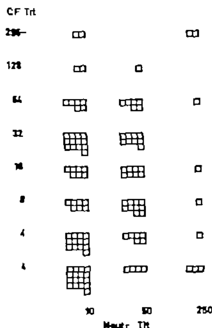


Fig. 2. Relationship between neutralizing and CF titers, all positive sera included. Titers are recorded as reciprocals of final serum dilutions.

taining intranuclear inclusion bodies with margination of chromatin and halo formation. Tests with human standard positive immune serum yielded neutralizing reactions. Serologic investigation of the patient showed a CF titer of 32 and a neutralizing titer of 50

Discussion

According to the results presented above, obtained from serologic and virus isolation studies, cytomegalovirus infections seem to occur frequently in a Swedish population. The high proportion of individuals giving positive serological responses corresponds well with findings from other remote geographic areas [11]

It should be mentioned that the pattern of immune-response in this material has been studied in tests against the "Ad. 169 cytomegalovirus strain. Furthermore antibodies against the "Ad. 169" strain were also found in serum samples from the infant excreting the "Ström strain. A relationship between the American and the Swedish agents is clearly indicated but antigenic identity has not yet been proved. Weller & Hanshaw [16] have claimed, that the cytomegaloviruses should be considered as a group of related antigenically not homogeneous, agents

The unique host parasite relations of cytomegaloviruses and the peculiar attributes of these agents have also in this work caused practical difficulties of the kind described by earlier workers in this field [16] Thus, it was impossible to prepare reference antisera in laboratory animals. It was also difficult to accomplish serial propagation of early virus passages and there was furthermore a low stability in titers of virus and antigen pools.

The serological responses distributed by age groups gave a curve not completely similar to the typical pattern of immune response to the common communicable virus infections in man. Thus, for instance the cytomegalovirus antibody never reached the incidence of almost 100 % in adults. On the other hand the findings resemble the patterns found with herpes simplex infection. It has been postulated that cytomegalovirus persists in the body as is true of herpes simplex virus. There was in addition no evidence of a decrease in the number of positive individuals between 6 and 24 months of age On the contrary an increase was recorded. If this finding proves significant it indicates the common occurrence of cytomegalovirus infections in this age group

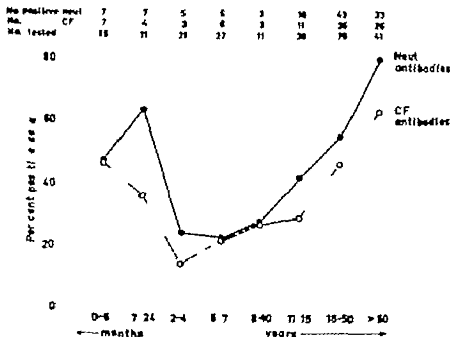


Fig. 1. Distribution of neutralizing and CF antibodies in different age groups of [illegible] children and adults. Neutralizing antibodies tested at final serum-dilution of 1/10. CF antibodies at a final serum-dilution of 1/4.

Isolation of cytomegalovirus

The demonstrated incidence of antibodies in children and adults of different ages indicated that cytomegalovirus infections often occurred in a Swedish population. Therefore efforts were made to isolate virus from infants treated in the paediatric department with the tentative clinical diagnosis of cytomegalic inclusion disease. The efforts soon yielded positive results, cytomegalovirus being recovered from a 4-month-old infant. Detailed clinical and cytological data on this case will be given elsewhere. Human embryonic skin and muscle cell cultures inoculated with a mouth swab sample from this patient developed specific cytopathic changes in 10 days ("Ström strain"). Small foci of enlarged round cells appeared with a slow progression in the fibroblast outgrowth. Except for deposition of granular material only minimal changes were observed for a couple of weeks. After 4 weeks the characteristic changes had progressed somewhat and passages were made with supernatant culture fluid containing whole cells, released by scraping or by treatment with trypsin. After 4 passages the incubation period was shortened to 2 days with complete degeneration occurring within 14 days. From this passage on virus was present in the centrifuged supernatant fluid, and the titer of virus in fluids from the 5th passage was $10^{-4.3}$/0.1 ml.

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Department of Clinical Bacteriology
Section of Virology
Karolinska Sjukhuset
Stockholm 60
Sweden

In Fig. 1 a good correlation is shown between neutralizing and CF antibodies. This circumstance supports the assumption that the methods for demonstrating the presence of cytomegalovirus antibodies have been reliable. The quantitative correlation between CF and neutralizing titers is more difficult to evaluate (Fig. 2).

Virus isolations may have a limitation as diagnostic procedure as a high percentage of healthy children are cytomegalovirus excretors [12]. Also, CF tests have been reported negative in infants with typical cytomegalic inclusion disease [10]. Assessment of the significance of infection with cytomegalovirus is difficult. Further investigations are therefore needed to delineate the syndrome of cytomegalic inclusion disease. Healthy babies and suspected cases are now subjected to clinical pathologic and virologic studies.

Summary

Serum samples from 24.. children and adults of different ages in a Stockholm population were tested for neutralizing and CF antibodies against cytomegalovirus. Antibodies were frequently found in newborn infants. A rather small number of children between 2 and 4 years of age showed antibody. This number slowly rose during childhood, adolescence and adult life approaching an incidence of 80 % in individuals more than 50 years old. Cytomegalovirus was isolated from a mouth swab specimen of a 4-month-old infant with the clinical diagnosis of cytomegalic inclusion disease.

Acknowledgements

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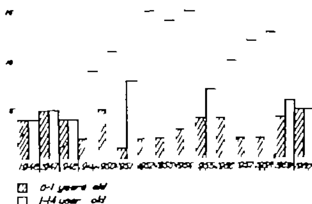


Fig. 1 Time distribution.

mal frequency There were 4 cases of mature birth. Possible primary infection ld be traced in 27 cases, partly from the e history and partly from the mother

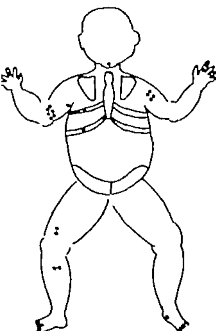


Fig. 2. Localization of the infection.

st tement in conference with the re-investigator It consisted of upper respiratory infection, chiefly purulent rhinitis, in 13 cases; paronychia in 5 cases; pyodermia in 3 cases; umbilical infection in 3 cases; gastroenteritis in 2 cases and otitis in 1 case The growth of st phylococcus aureus was found in all the 18 cases where the pus was cultured from the incision or puncture made In 10 of the 18

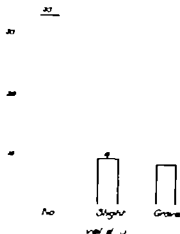


Fig 3. Results of the re-examination.

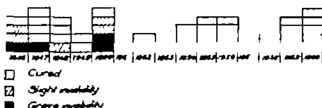


Fig. 4. Results of the re-examination correlated with the year of falling ill.

cases the bacterial strains were sensitive to all antibiotics. In 5 out of the remaining 8 cases penicillinase-producing staphylococci were found, and in 3 cases the strains were less-sensitive to penicillin *in vitro*. Penicillin had been given to 6 out of these 8 patients before making the culture. Of the 8 patients with infection of penicillin resistant staphylococci 3 came from another hospital, one showing infection with staphylococcus of phage type 47. In 3 patients the infection was a postoperative complication at one of the clinics for paediatric surgery. Of the remaining 2 patients 1 showed an infection contracted at yet another paediatric clinic and 1 an infection from birth. In 10 out of the 29 newborns infections were found in the ...n of purulent rhinitis, or paronychia from their stay in the maternity wards. Cultures and strain resistance patterns were made for 6 of them and all showed staphylococci sensitive to penicillin.

Only local symptoms without fever were present in 34 cases; local swelling or limitation of an extremity's function and movement led to the first consultation in most cases. Septic manifestations, such as meningitis, peritonitis, pericarditis and jaundice or bleeding were not present in any of the cases. Specific infections as tuberculosis, syphilis or typhoid do not appear in the material.

Penicillin was given in 37 cases, sulpha in 4 cases and broad-spectrum antibiotics (after determination of the strain resistance pattern) in the remaining 8 cases. Average duration of treatment was 5 weeks. Metastatic

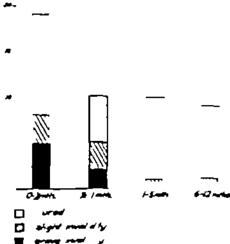


Fig. 5. Results of the re-examination correlated with the age of falling ill.

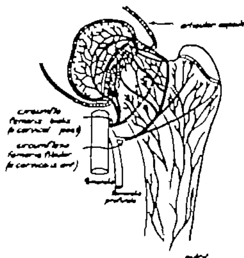


Fig. 6. The metaphyseal and epiphyseal blood supply in childhood. After Nussbaum [17].

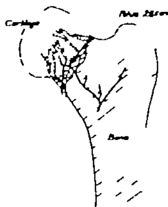


Fig. 7 The epiphyseal blood supply before the ossification of the epiphysis. After Mattick [15].

abscesses after the initiation of the treatment were found in 4% of the cases. Surgical therapy was largely conservative. Local abscesses requiring incision were found in 12 patients. Pathological fracture appeared in 2 cases. Clinical diagnosis of purulent arthritis was made in 12 cases. Sequestrotomy was performed in 4 cases. Secondary corrective operations consisted of elongation or key-osteotomy in 4 cases and arthrodesis in cases.

Forty-three of the patients were re-examined with an observation time varying between 2 to 17 years. Three children had died, of whom 1 died during treatment and 2 from other causes sufficiently long after recovery to make confirmation of the treatment result from the accessible data possible. Three could only be verbally contacted but were said to be completely trouble-free.

Results

The mortality was 1 in 49 cases, or 2%.

During the year 1952 1 patient contracted osteoarthritis of the hip-joint at the age of 1 month, and died at the age of 2 months while under treatment. The immediate cause of death was a metastatic focus at the base of the skull close to the foramen magnum, with compression of the medulla oblongata and purulent meningitis. The primary focus was localized in the proximal end of the femur. The diagnosis was made 1 week after admission and the occurrence of a complication with arthritis of the hip-joint. The possibility of traumatic epiphyseolysis during delivery had created difficulties in differential diagnosis. The late bacteriological diagnosis which led to secondary sepsis combined with the unfortunate localization of the metastatic focus is considered to be responsible for the fatal outcome.

The re-examination covered clinical and radiological evaluation. Cases with slight irregularities in the epiphysis without subjective trouble or additional objective consequences, have been regarded as cured in spite of the contingency of arthrosis in later life.

Recurrence or development into chronic infection did not take place. None of all those re-examined revealed susceptibility to infection later in life and apart from the sequelae of osteomyelitis all were completely healthy. The results are demonstrated in Fig. 3. From Fig. 4 it is evident that the treatment's results have

TABLE 1

Cases

Marked invalidity

4 Osteoarthritis of the knee
2 Osteoarthritis of the hip
1 Osteoarthritis of the knee and of the hip

Total 7 cases

Slight invalidity

2 Osteoarthritis of the hand or foot
1 Osteoarthritis of the elbow
1 Osteoarthritis of the shoulder
2 Osteomyelitis of the proximal end of the humerus
1 Osteomyelitis of the proximal end of the tibia

Total 8 cases

TABLE 2

	Age of falling ill	Present age	Result
Osteomyelitis of the proximal end of the femur			
M. Y ♂	0 days	8 yrs	Cured
J K. W ♂	5 days	13 yrs	Invalid, cm shortening
A. W ♂	14 days	16 yrs	Invalid; 7 cm shortening
T H. ♂	14 days	13 yrs	Invalid; 8 cm shortening
M Y ♂	4 months	3 yrs	Cured
T W ♂	11 months	17 yrs	Cured
Osteomyelitis of the distal end of the femur			
J K. W ♂	5 days	13 yrs	Invalid; 7 cm shortening
I L. R. ♀	10 days	5 yrs	Slight obj changes
S Å o	11 days	16 yrs	Invalid; 22 cm shortening
U K. H ♂	13 days	5 yrs	Reckoned invalidity
C. M. B ♂	16 days	6 yrs	Reckoned invalidity
M. L. ♀	3 weeks	9 yrs	Invalid; 12 cm shortening
I B ♀	6 weeks	16 yrs	Cured
T B ♂	1 month	15 yrs	Cured
O y ♂	2 months	2 yrs	Cured
K. Y ♂	[illegible] months	3 yrs	Cured
P J K. ♂	9 months	14 yrs	Cured

not varied significantly during the current 15-year period Thus 33 patients (68 %) showed neither subjective nor objective clinical trouble nor radiological bone changes of any significance at the time of the follow up examination. Correlation of the re-examination s results with the age of falling ill (Fig 5) shows that the corresponding figures for complete restitution in the newborn (below 1 month of age) are 16 out of 29 patients (55 %) and in infants (1–12 months of age) 17 out of 19 patients (89 %)

If the results are correlated with the localization (Table 1) it is evident that all the 7 patients with marked invalidity had suffered from an osteomyelitis with primary focus in the distal or proximal end of the femur and complicating arthritis of the knee or hip-joint Five out of the 8 patients with slight objective consequences of their illness had suffered from verified osteoarthritis of the hand, or the foot or the shoulder joint or the elbow

A closer study of all cases with a primary focus in the proximal or distal end of the femur (Table 2) illustrates the difference between the prognosis for the newborn and that for the infant

Discussion

The comparatively poor results in the new born is in accordance with the only comparable re investigation, namely that of Lindell & Parkkulainen [14] These authors found remnants of slight bone damage and completely unimpaired function in 50 % in the case of the newborn and in 81 % in that of infants (1–1[illegible] months of age) They suggest that the poorer results in the newborn depend on the greater frequency of penicillin-resistant strains in them than in older children.

According to the present material the prognostically most significant factors are the age of falling ill and the localization of the infection. The complicating purulent arthritis which seems to occur among chil dren under 1 month of age explains the bad prognosis of acute osteomyelitis in early infancy

Primary localization in the metaphysis in osteomyelitis during childhood has been explained by the lively blood flow in this area together with the slowing-down of the blood-stream in the end-arteries followed by wide venous sinusoids which exist close to the epiphyseal line throughout the growing period [12, 13 16, 17 18]. Where abundant anastomosis can be seen (in the diaphysis and the epiphysis) the blood is thought to combat the infected thrombus more easily

It has been pointed out [1 4] that the joint capsule fastens on to the metaphysis in the hip and the elbow joints whereas in the other joints the met physis lies extra-articularly This should lead more easily to arthritis in the case of primary metaphyseal osteomyelitis around the hip and the elbow Other authors have shown how vessels (although sparsely) penetrate the epiphyseal line and thus convey the infection from the metaphysis to the epi physis in the age-group where the epiphysis is mainly composed of cartilage These per forating vessels have been angiographically studied by Trueta [1] However as early as 1898 Dardenne–Allbert [6] and Herzog [10] stated that osteomyelitis with primary localization in the epiphysis dominated among young infants. Whether the epi physeal line contains perforating vessels or not or whether the joint capsule fastens on to the metaphysis or not thus appears to be insignificant in this age-group. It seems more important to find out why osteomyelitis with primary localization in the epiphysis should appear in infants while it so seldom appears in older children. Lexer [13] showed how different parts of the bone were affected at different ages and wished to relate this to the different vascular conditions at different ages Later angiographic studies, principally to be found in anatomical literature confirm this proposition.

The only true blood supply to the epiphysis in the femur proximal and distal end in children consists of vessels in the visceral blade of the joint capsule [15 16, 17 22] (see Fig. 6). Microangiographic studies of the vessel's development in the epiphysis f the proximal end of the femur from the foetal stage t the age of 15 years [15, 22] show how the epiphyseal vessels consist of end-arteries before ossification of the epiphysis (see Fig 7) Here too, as in the metaphysis of the growing child, there is a discrepancy between the diameter of the end-arteries and the veins which should lead t a slowing-down of the blood stream. Not until the ppearance of ossification centres do anastomoses appear between the epiphyseal vessels which might hinder bacterial growth here during sepsis.

Thus, before the ossification of the epi physis there is the theoretical possibility of a predilection for primary infection of the epiphysis with a great risk of purulent arth- ritis. This has also been proved through autopsy findings in early reports [10, 19]. At best only a partial disintegration of the carti lage-composed epiphysis will take place with future limit tion of growth and deformation.

In this material osteoarthritis appeared even before osteomyelitis could be cor rectly diagnosed and the only possibility of further combating this prognostically serious condition ppears to be through vigilance against the disease and through

prophylactic measures. Vigilance leads to an early bacteriological diagnosis through blood-culture and local test puncture. It leads to an early suspicion of complicating arthritis and to earlier adequate therapy. In all the cases of osteoarthritis of the hip-joint in this material the correct diagnosis was made 1 week to 10 days after consultation with a doctor. Moreover it appears as if the tendency in recent years has been that of dependence on antibiotic treatment alone, thus often entailing the neglect or procrastination of arthrotomy. Before the advent of antibiotics, arthrotomy (broad incision of the articular capsula followed by skin-sutures) after a verified diagnosis through puncture was performed in most cases. In Karlström's report [11] 15 out of the 19 cases of purulent arthritis in infants had normal movement and good function upon reexamination (the 4 with hip-joint purulent arthritis were invalids). Some of his contemporary authors do not have equally good results; arthrotomy is not sufficient to hinder the great percentage of invalidity. In the eventuality of complicating arthritis antibiotics seem only to have altered the prognosis *quoad vitam*. For example in the present material 5 out of the 11 patients with osteoarthritis of the knee joint were invalids upon re-examination. This can be compared to no invalids out of 7 with osteoarthritis of the knee joint in Edberg's [7] and Karlström's [11] reinvestigations in 1913 and 1935 at The Kronprinsessan Lovisa's Children's Hospital. By combining intensive antibiotic therapy with early arthrotomy or repeated punctures it appears possible to improve the prognosis of purulent arthritis in infants. Several authors recommend local antibiotic therapy as well. Clarke [4] gave erythromycin in glycerin in order to raise the local concentration. Cases of arthritis of the hip-joint for example must be immobilised in optimal position with maximal abduction in order to minimize the tendency towards dislocation. "Aspiration, abduction, appropriate antibiotic" [8] is a good rule to remember. If marked swelling and induration is present then punctures without arthrotomy will probably not prove adequate.

Prophylactic measures principally imply measures against staphylococcus infection in children below 1 month of age. A more continual control of infections during the first month after discharge from the maternity ward seems desirable. The discovery that nosocomial infections can be contracted at the maternity wards but show an incubation period of up to one month [3] means that there could have been more cases than is indicated here of primary infection from the maternity wards.

The increased incidence of infections at the maternity wards, often with *in vitro* penicillin resistant strains, lacks support in our investigation but has been reported [3]. This study does not suggest that the high frequency of penicillin-resistant strains is the predominant reason for the poorer results of osteomyelitis in young infants than in older children [14]. The complications propounded by Cullen & Hargadon [5] in support of the difference between initial penicillin and broad spectrum antibiotics, namely abscess, sequester and pathological fracture, are not verified as being dependent on the efficacy of the particular antibiotic therapy chosen. In the present material the cases with

abscess, sequester or pathological fracture as the only complication were cured through prolonged and intensive penicillin therapy and minor operations. The small number both of secondary inflammatory foci—2 cases—(cf. Blanche [4] 1035–1050 in 50 %) and of other septic manifestations, speaks for a high percentage of sensitivity towards penicillin in vivo. The efficacy of the antibiotic employed should be judged by its ability to limit the infection, as has also been pointed out earlier by Gernet-Smidt [9]. With regard to the high incidence of penicillin resistant strains in the maternity wards (40 % [3]) and to the rapid progress in children under one month of age methicillin initially is strongly recommended. In older children without clinical signs of sepsis and in a good general condition penicillin seems to be sufficient while awaiting determination of the resistance pattern.

Summary

Forty-nine cases of radiologically confirmed acute haematogenous osteomyelitis in infants under one year of age during the period 1946–1960 were reinvestigated. Neither the morbidity nor the result varied during the 15-year period. The mortality was 1 in 49 cases. Thirty three cases showed neither subjective nor objective trouble nor any significant radiological bone changes. Eight showed slight and 7 marked invalidity. Recurrence or development into chronic infection did not take place. The age of falling ill and the localization of the infection is of great importance to the prognosis of the disease. The prognosis is worst during early infancy when the epiphysis is not ossified and is theoretically liable to primary infection with a great risk of purulent arthritis. Vigilance against the disease, early *adequate treatment of complicating arthritis* and prophylactic measures are recommended.

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Department of Paediatrics
Kronprinsessan Lovisas Barnsjukhus
Polhemsgatan 30
Stockholm K
Sweden

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From the Department of Pediatrics, University of Umeå Medical School, Sweden

The Xylose Absorption Test in Infancy

by GUNNAR W. MEEUWISSE and GEORG DANO

The xylose absorption test in the diagnosis of malabsorption disorders is drawing increasing attention, as witness numerous publications on its clinical use [1–8, 11, 13, 15–18, 20, 22, 23, 25, 30, 32]. Searching this literature some disagreement about the criteria for the evaluation of xylose absorption can be noted. This is, with some exceptions [2, 5, 7, 22, 25], at least in part due to the use of too small materials.

Until recently the literature on the xylose absorption test gave very scarce information on normal standards for children (especially infants). There is still no established standard procedure for the performance of the xylose test in these ages [3, 13, 15–18, 20, 22, 33]. In an attempt to find a simple procedure for the xylose absorption test in children, we have studied the relationship between the blood concentration and urinary excretion after oral administration of xylose to normal infants. In addition one case of celiac disease was studied.

Physiology

Xylose[1] is a watersoluble pentose not present in appreciable amounts in the blood or urine of the fasting individual. Normally in the adult the greater part of an orally administered dose of xylose (25 g) is absorbed by the small intestine. Percentages between 65 and 100% have been reported [4, 16, 19]. Following a rapid intravenous injection of xylose the blood concentration—after a short distribution phase—falls as an exponential function of time with a k value of about 1.0% per minute corresponding to a halflife of ~70 minutes [26, 33]. After 5 hours all but 6% of the injected amount has been eliminated. The urinary xylose excretion is directly proportional to the blood concentration. Part of the xylose is metabolized. As the two modes of elimination together result in a xylose disappearance proportional to the blood concentration, this proportionality must also hold for the metabolic utilization. In other terms there is a constant relationship between the variables: blood concentration, metabolic utilization and urinary excretion. The xylose excretion is independent of the diuresis, but it does depend on the glomerular filtration rate [12]. Some of the filtered xylose is reabsorbed. The reabsorption, which is independent of the plasma concentration, amounts to about ¼ of the filtered xylose [27, 28]. Hence, the xylose clearance will be about ¾ of the inulin or endogenous creatinine clearance. The percentage of infused or absorbed xylose recovered in the urine varies; the route, by which xylose is administered, may make some difference [5]. Bypassing the liver by intravenous (systemic) administration probably delays the metabolic elimination, exerted by the liver. The differences, however, appear to be small and are not accounted for in this report.

[1] In this article xylose stands for $d(+)$-xylose

TABLE 1 *Some data on the group of "normal" subjects studied with the aid of simultaneous blood and urinary xylose curves*

Subj No.	Sex	Age months	Weight g	Xylose dose	Cause of hospitalization
1	male	1½	4200	3.6	Illness of mother
2	fem.	1½	4600	3.5	Idiopathic hypoglycemia
3	fem.	2	5200	4	Panaritium of a toe
4	fem.	2½	5100	4	Hip joint luxation
5	male	7	7800	6	Hydrocephalus
6	male	9	8500	6	Atopic dermatitis
7	fem.	14	9500	9.5	Atopic dermatitis

even in the same individual [19 33]. On an average it is 44% in healthy adult subjects [5, 19]. The percentage increases, of course with increasing glomerular filtration, but it decreases with increasing metabolic utilization.

Material

In one group individual blood and urinary xylose curves were estimated. This group consisted of 7 "normal infants (Cases 1 7), i.e the infants were healthy or had been cared for in the hospital for certain diseases, not affecting the gastrointestinal tract or the kidneys (see Table 1) In addition one child (Case 8) suffering from celiac disease was studied in the same way This was a 6-month-old girl, weighing 6000 g The fat absorption—in this case before treatment with a gluten free diet—was 75%, as estimated by a 4-day fat balance study Besides this group another group of 1 "normal" children, aged 1–14 years, were studied in the traditional way just to obtain the 5-hour urinary excretion value

Methods

The subjects were fasted for about 8 hours prior to the administration of the xylose test dose this amounted to 15 g per sq m of body surface administered as a 10 % solution in water The absolute amounts in Cases 1–7 is given in Table 1; in Case 8 the dose was 4 5 g After 3 hours the subjects were given water ad libitum. After 5 hours they received their ordinary meals, but fruits were excluded, because of their xylose content The subjects were in bed during the whole test They were sedated, when necessary with 0.5–1 0 chloral hydrate rectally The urine was collected in one-hour samples for 8 hours by means of a catheter à demeure The subjects 5, 6 and 8 were studied for 10 hours. At hourly interval the bladder was rinsed with 10 ml sterile isotonic saline which amount was added to the sample Each hour usually in the middle of the urine periods, 0.. ml capillary blood was taken and deproteinized according to Somogyi [40]. Xylose in blood and urine was estimated by a modification of the method of Roe & Rice [21 4]

Results

The results are presented in Fig 1 The curves representing the blood xylose levels (in mg per 100 ml) and the hourly urinary xylose excretion (in percentage of the test dose) are running a similar course The delay between the time of urine production and its appearance in the bladder—approximately 3 minutes—has been disregarded For the sake of simplicity the amount of xylose excreted each hour is recorded on the abscissa at the midpoint of the sampling period [1] A high blood

[1] It is recognized that this procedure gives

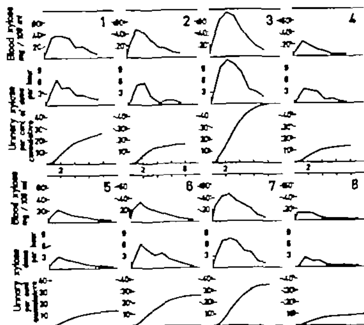


Fig. 1 Blood xylose levels and hourly and cumulative urinary xylose excretion in 8 infants after oral administration of d xylose (15 g per sqm body surface). The urinary excretion is expressed as percentage of the dose administered. The numbers in the graphs refer t the different case numbers (see Table 1); subject 8 suffered from celiac disease.

xylose level corresponds to a high urinary xylose excretion. Likewise a low blood level corresponds to a low urinary excre tion. Maximum blood levels were reached after 1½ to ½ hours. Then a slow decrease follows. Often a small discontinuity was observed after 5 hours (the time of feeding) The total percentage of the dose excreted from the beginning of the test up to the end of each hour (cumulative excretion) is also represented in Fig 1 These curves are approximating their asymptotes at about 8 hours, a fact, best seen when the experiment was extended to 10 hours (subjects 5 6 and 8) For comparison with xylose excretion values, published by others, Fig _ shows percentages of 5-hour urinary xylose excretion, derived from the tests in the subjects 1–7 This figure also includes the 5-hour excretion values of the 12 children obtained in the traditional way The lowest values for normal subjects —10.5 and 11.5% excretion in the subjects 5 and 4 respectively—were found in the

values, that are not quite representative for the xylose excretion per unit of time at the midpoint of the periods. Near the base of the curve they often are little too high and near the top they often are a little too low A more accurate curve of the xylose excretion per unit of time can be derived from the smoothened curve representing the amount of cumulati xylose excretion, say function $\varphi(t)$, (Fig 1). The first derivative of $\varphi(t)$, $\varphi'(t)$ is the actual xylose ex cretion per unit of time t the time t As $\varphi'(t) = d\varphi(t)/dt$, one only has to measure the slope of the curve for the cumulative xylose excretion at the time t, to find the actual xylose excretion per unit of time at the time t. Curves constructed in this way gi only small deviations from those presented in Fig 1.

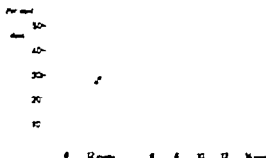


Fig. — The 4-hour urinary xylose excretion in 19 infants and children of different ages, without known malabsorption or renal disorder.

infant group but in the same age group also a high value was recorded, 41 % in Case 3. The xylose excretion in the child with celiac disease (subject 8) was 7.8, 9.0, 9.3 and 9.4 % at 5, 8, 10 and 24 hours, respectively.

Nearly all infants and small children passed one or more loose stools during or after the test. In some cases with low xylose excretion this happened already at 1 or 2 hours after the ingestion of xylose.

Discussion

Size of the test dose

For adults a xylose test dose of 25 g has generally been adopted. Doses of 5 g are claimed to give comparable results, though the percentage excreted xylose in 5 hours is somewhat higher both in normals and in patients with malabsorption [5, 25]. This five-fold reduction of the dose has recently been criticized, as it results in greater overlapping between the xylose excretions in normals and abnormals [11].

In children there is less uniformity in the size of the applied test dose. Benson *et al.* [3] recommended a dose of 0.5 g per pound of body weight with a maximum of 25 g. Jones & di Sant' Agnese [16] used only about half of this dose—0.5 g per kg of body weight—and they report that doses of 1 g per kg body weight were tried, but led to transient mild diarrhoea and greater variability in results. Lanzkowsky *et al.* [17] used 0.3 g per kg body weight with a maximum of 5 g. Loran & Uyeyama [18], studying a group of infants and children between 2 months and 1 years of age, used a standard dose of * g. The statement by McCrae [*0], that in normal subjects the percentage absorbed xylose is not influenced by the size of the test dose does not seem to be correct. Small doses have been found to be more completely absorbed than large doses [5, 6, 25]. Therefore it is necessary to adapt the dose to the size of the child, when the results of the xylose absorption test in different ages are to be compared. The most physiological reduction of the dose seems to be in relation to the body surface ratio. Hence we used the doses

$$\frac{\text{body surface in sqm}}{1.73} \cdot 25\text{ g}$$

$$\approx \text{body surface in sqm} \cdot 15\text{ g}$$

The same dose, 15 g per sqm body surface was recently used by Ingomar *et al.* [15]. It differs negligibly from the doses used by Polonovski & Gombault [22] and Ferrier *et al.* [7] to children above the age of 18 months—14.5 g/sqm body surface. Their publications give at present the best information on normal xylose excretion values in children. These doses lie somewhere between those of Benson *et al.* and those of Jones & di Sant' Agnese. They are more than twice as big as those used by Lanzkowsky *et al.*

The above-mentioned reduction formula facilitates the calculation of the renal xylose clearance per 1.73 sqm of body surface from the data, reproduced in Fig. 1. These values may then be directly compared with other renal clearances, which usually are expressed in the same way.

Renal clearance and metabolism

The similarity of the shapes of the curves for the blood xylose concentration

TABLE 2. Renal xylose clearances and derived glomerular filtration rates in subjects 1–8

Subject No.	1	2	3	4	5	6	7	8
Renal xylose clearance (ml/min/1.73 sq.m)	60.7	37.5	62.2	72.0	61.3	76.3	68.2	7_.4
Glomerular filtration rat (ml/min/1.73 sq.m)	77.8	48.1	79.7	92.3	78.6	97.8	87.4	92.5

and the urinary xylose excretion per unit time (Fig 1) makes evident that the degree of xylose absorption can be judged from the urinary xylose excretion, equally well as from the blood xylose level. Their relationship can be expressed as follows:

$U\ V = P\ C$ or by integration.

$$\int_0^t U\ V = \int_0^t P\ C = C \int_0^t P_{dt}$$

if C is a constant

(U = urinary xylose concentration. V = urine flow per unit of time P = plasma xylose concentration—in man the same as the concentration in whole blood. C = renal xylose clearance $\int^t U\ V$ = the cumulative xylose excretion up to the time t. $\int_0 P$ = the area under the curve for the blood level between the time 0 and the time t.

The renal xylose clearance can be calculated, if the urine sampled until a certain time limit, is analyzed for xylose (cumulative excretion) and the blood xylose level is estimated with suitable intervals until the same time limit (Table 2). In our study the xylose clearance was directly expressed as ml per minute per 1 73 sqm of body surface by the formula.

$$C = \frac{\text{per cent excr}}{\int^{490} P} \cdot 5{,}000$$

Here P is the blood concentration in mg per 100 ml.

In adults a figure of 0 8 has been obtained for the xylose inulin ratio. If this figure is applied to the present material approximate values for the inulin clearance can be derived from the xylose clearance values by introducing a factor of 1.28 (the reciprocal of 0 78). The values thus obtained (Table 2) are consistent with the normal values for glomerular filtration, amounting from 46–79 ml at 1½ months to 74–123 ml per 1 73 sqm at 14 months of age (range for $\pm 2\sigma$) [31]. The results of these calculations indicate, that a figure of 0 78 for the xylose/inulin clearance ratio can be accepted also in infancy

Influence of glomerular filtration rate

It is well known, that infants and small children—as compared with adults—possess relatively slow glomerular filtration rates. The fraction of absorbed xylose excreted in the urine will therefore be small in healthy infants just as in the adults with renal disease One might suspect, that the low xylose recovery

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The integral of the blood xylose concentration was calculated from the area under the curve, using Simpson rule one nine ordinates, giving the total area of eight strips with width of 60 min for total period of 490 min.

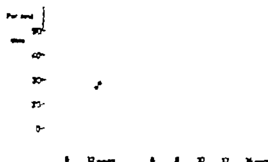


Fig 2 The 5-hour urinary xylose excretion in 19 infants and children of different ages, without known malabsorption or renal disorder

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Renal clearance and metabolism

The similarity of the shapes of the curves for the blood xylose concentration

TABLE 3. *Xylose absorption* (%) *as calculated from the mean 5-hour xylose excretion in different ages* (Data taken from Lanzkowsky *et al* [17])

The total xylose excretions (corrected values) and the actual excretion fractions have been estimated with the aid of the mean glomerular filtration rates of the specified age groups.

Age (years)	0–½	½–1	1 3	3–10	10–16	Adult
Mean 5-hour xylose excretion (%)	16.7	23.3	25.5	34.2	36.7	36 1
Total xylose excretion (%)	20 9	29.8	28.3	35.0	42.6	40.1
Actual excretion fraction	0.33	0.35	0 43	0.44	0 44	0.44
Xylose absorption (%)	63	78	67	80	93	91

less, the figures in T ble 3 indicate that the xylose absorption is virtually lower in infants than in adults. This explanation of the lower urinary xylose recovery rates in infants was also adopted by Lanzkowsky *et al.* [17]. They considered, however that these in part also were due to the smaller urinary volume, found in infants and young children as compared t adults. This argumentation does not seem to be correct. In the first place: the urinary volume in infants is not smaller in infants than in adults, when correlated to weight or body surface Second: the xylose excretion is basically independent of the diuresis [9], although the diuresis may be somewhat ugmented by increased osmolar load, when large amount of xylose are passing through the renal tubuli. It is true, that the xylose absorption increases with increasing age (cf. Table 3) as do the daily urine volume and many other variables in the growing child. The coincidence is quite natural and there is no reason to suppose a causal relationship

Duration of urine sampl ng

From the curves for the cumulative xylose excretion, it can be concluded, that urine collection for about 8 hours yields almost the total (>95 %) urinary xylose excretion. Extending the period of urine collection for one or more hours has evidently little influence on the figure for cumulative excretion. E.g subject 5· 1 .9 and 13 4 % were recovered up to 8 hours and 10 hours, respectively The corresponding values for subject 6 were 25.6 and 26.4 %. In the patient with celiac disease (subject 8) 9.0 and 9.3 % were obtained at 8 hours and 10 hours respectively In order to achieve an index for the *total* absorption of an orally administered xylose dose, a reasonable accuracy will therefore be obtained, if the urine is sampled for at least 8 hours There is no need for a catheter provided that the first miction, observed after 8 hours is included.

Deviation from normal in malabsorption

Two normal subjects of comparable age (subjects 3 and 4) showed a great difference in their 8-hour excretion values 50 and 13 4 %, respectively This is in agreement with results reported in the literature [7 15, 17 22]. This broad variation and the small difference observed between the values of the patient with celiac disease and a normal child of corresponding age (subjects 8 and 5 with 8-hour excretion values of 9.0 and 12.9 %, respectively) indicate that a distinct separation between normal subjects and malabsorption cases can hardly be

expected. The overlap is larger in children than in adults [6 11 15 22].

It is possible that in normal subjects the range of variation can be narrowed by altering the size of the test dose. This would be advantageous only if simultaneously the border between the normals and the patients with malabsorption becomes more distinct. The dosage used in our study often caused fast intestinal passage and in the infant group one or more loose stools, indicating incomplete absorption, were always produced. Such an increased peristalsis may be responsible for a worse absorption [30], although it is just as likely that the causality is the reverse. Furthermore, the xylose *concentration* may influence the peristalsis and thereby affect the results. In the present investigation, as in many others, a 10% watery solution was used, while 4.6% is isotonic. The optimal dose and concentration of xylose for its use as a test on the intestinal absorption capacity is still not known. Physiological studies on gastric emptying suggest that solutions nearer isotonicity might be more readily and perhaps more constantly absorbed than concentrated ones [14].

Until more experience with the xylose absorption test in infancy and childhood has been gained, it is advisable to estimate both the urinary excretion of xylose and the blood xylose concentration. In an early paper in this field the blood xylose concentration was used as a criterion on the intestinal absorption capacity [32]. In a recent publication the advantage of measuring the maximal blood concentration instead of the urinary excretion has been demonstrated [15]. Beck *et al* [2] report the best separation between normal adults and patients with malabsorption, when the ½ hour or 1 hour blood xylose concentrations rather than the maximal xylose concentration are taken as criterions. They considered, logically, the early blood xylose concentration to be more a measure of xylose absorption *rate* the 5-hour urinary excretion being more a measure of the *total* absorption. The early blood concentrations are less influenced by individual variations in the glomerular filtration rate than are the later blood concentrations (peak values) and, of course, the urinary excretion. Though relatively large test doses were used in our subjects the blood concentrations often remained below normal adult values (>15 mg% at ½ hour and >30% at 1 hour). Hence also with respect to this criterion, special, pediatric standards have to be developed.

Summary

The blood xylose levels and the urinary xylose excretion were studied at hourly intervals for 8 or 10 hours after an oral dose of d-xylose to 7 normal infants and 1 case of celiac disease. A close correlation was found between the blood and the urinary xylose curves.

For measurement of the total xylose excretion in infancy it was found necessary to extend the period of urine sampling to at least 8 hours. Further sampling beyond this limit was of no practical importance.

The values of xylose excretion obtained in the normal subjects were often lower than those reported in adults. The range of variation was relatively broad. The influence of the glomerular filtration rate on the xylose clearance is discussed. It is concluded, that the relatively lower urinary xylose recoveries in infancy cannot solely be due to a lower rate of

glomerular filtration, but must in part also be due to a smaller intestinal absorption capacity as compared with adults. Determination of early values of blood xylose concentration as criterion of the xylose absorption rate is discussed.

Normal values for the xylose absorption test in pediatric age groups have to be worked out with standardized conditions such as duration of fasting (prior to and during the test) position and activity during the test, size and concentration of the test dose length of the urine sampling period and time interval of blood sampling after administration of the test meal.

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Department of Pediatrics
University of Lund
Barnsjukhuset
Lund
Sweden

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From the Department of Anatomy (Head: Prof. Ture Petrén) Karolinska Institutet and the Department of Metabolic Research (Head. Dr Harry Boström) Wenner-Gren Institut University of Stockholm, Sweden

Teratogenic Action of Salicylates Related to the Inhibition of Mucopolysaccharide Synthesis

by K. S. LARSSON and H. BOSTRÖM

Introduction

Several antiphlogistic drugs, such as cortisone salicylates and phenylbutazones, have been shown to depress the synthesis of mucopolysaccharides in mesenchymal tissues [5 6, 8, 11 20 2., 25].

Many investigations also indicate that acid mucopolysaccharides play an important role in embryonic development and differentiation. Thus, several studies have shown embryonic tissues to have a high mucopolysaccharide content and, furthermore, isotope experiments with S^{35} labeled sulphate as tracer have indicated a high sulpho-mucopolysaccharide synthesis during embryogenesis [1 3, 4 7 10 15 .1].

The possible consequences of an interference with acid mucopolysaccharide synthesis during a certain stage of embryological development has also been illustrated in earlier studies from our laboratories on cortisone-induced cleft palate in mice [16 17]. Another example of such interference has been reported by Larsson *et al* [18 19] concerning the salicylate-induced skeletal and vessel malformations in mice

To obtain further support for the theory that depression of acid mucopolysaccharide synthesis has a teratogenic action [17 18] we studied 3 members of the salicylate group in this respect. The compounds used were the sodium salt of salicylic acid and of its therapeutically inert isomer p-hydroxybenzoic acid, and the sodium salt of acetylsalicylic acid.

Material and Methods

Pregnant primiparous mice of A/Jax and CBA strains were used. They were mated as described elsewhere [15], and vaginal plug could easily be observed in most cases. This day was denoted as the zero day of pregnancy [15].

The sodium salts of salicylic acid, p-hydroxybenzoic acid and acetylsalicylic acid were given in doses of 10 mg/0.1 ml of distilled water A single intramuscular injection was given either on the 8th or the 12th gestation day The embryos were removed on the 18th gestation day and inspected for resorption and gross malformations. Living embryos from mothers injected

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TABLE 1 *Incidence of resorbed embryos of implanted embryos from mothers given 10 mg of one of the substances i.m. on the 12th gestation day and on the 9th gestation day. The mothers in the control group were untreated*

Substance	Strain	Resorption: 1*th gestation day	Resorption: 9th gestation day
COONa, OH (sodium salicylate)	A/Jax	41.3 % of 439 implanted	18.1 % of *"6 implanted
	CBA	4 4 % of 114 implanted	8.9 % of 184 implanted
COON, OH	A/Jax	12.8 % of 43 implanted	13.4 % f 89 implanted
	CBA	—	—
COONa, $OCOCH_3$	A/Jax	16.0 % of 163 implanted	12.0 % of 70 implanted
	CBA	10.3 % of 135 implanted	13.8 % of 151 implanted
Control group	A/Jax	10.6 % of 407 implanted	
	CBA	9 6 % of 166 implanted	

on the 9th gestation day were also stained with Alizarin red S for investigation of skeletal malformations [9 10]. Litters from non treated mothers were used as controls. The material includes the control group and the groups injected with the sodium salt of salicylic acid on the 9th and 1*th gestation day presented in earlier papers [18 19].

Results

Resorption

The incidence of resorbed embryos is expressed as the ratio of embryos, including all degrees of resorption, to the number of implanted embryos.

Sodium salicylate was the only drug to cause an increased incidence of resorption as compared to the control group. Furthermore this effect was limited to the A/J strain, with 41.3 % and 18 1 % resorption when the mothers were injected on the 12th and the 9th gestation day respectively. The incidence of resorption in the untreated control groups was 10 6 % in the A/Jax strain and 9 6 % in the CBA strain. The results are given in Table 1

Vessel anomalies

A particular type of gross malformation observed was vessel anomalies. They were located to the nose chin and paws. Histologically this malformation consisted of dilated vessel sacs [10].

The vessel malformations were found in 27 of **0 living embryos from A/Jax mothers injected with sodium salicylate the 1?th gestation day i.e. 11.9 ... 1 of 212 living A/Jax embryos from ...ers given ... ...lium salt of p-

hydroxybenzoic acid showed a similar malformation. This type of malformation was not found in any other experimental or control group The results are listed in Table 2.

Anomalies of ribs and vertebrae

Skeletal malformations of ribs and vertebrae consisting of changes in number and of pathological fusion of these bone anlagen, have been found to occur in a high incidence after injection of sodium salicylate on the 9th gestation day [19]

In the present investigation it was shown that of the drugs tested, only sodium salicylate caused a large increase in the incidence of both rib and vertebral anomalies in the A/Jax strain, 52.[illegible] % and 33.3 %, respectively In the CBA strain also, a fairly high incidence of rib anomalies, 16.7 %, was obtained. The incidence of these two types of skeletal anomaly is shown in Table 3

TABLE [illegible]. *Incidence of vessel anomalies in living embryos from mothers given 10 mg of one of the substances i.m. on the 12th gestation day and in embryos from the untreated control group*

		Vessel anomalies
COON, OH (ortho)	A/Jax	11.9 % of 226 living
	CBA	0 0 % of 169 living
COON, OH (para)	A/Jax	0.5 % of 12 living
	CBA	—
COON, $OCOCH_3$	A/Jax	0 % of 137 living
	CBA	0 % of 121 living
Control group	A/Jax	0 % of 254 living
	CBA	0 % of 150 living

Discussion

The results of the present study indicate that, within the salicylate group there is a reasonably good agreement between the teratogenic action of these drugs and their ability to depress the biosynthesis of acid mucopolysaccharides.

To illustrate the latter effect of the salicylate compounds tested, results of earlier isotope experiments [5, 6] are given in Fig. 1 *In vitro* experiments in which the relative rates of chondroitin sulphate synthesis were estimated by measuring the incorporation of radioactive sulphate into calf cartilage have been described [6] When salicylic acid was present in the incubation medium, a marked decrease in S^{35}-sulphate incorporation was noted in comparison with the controls The p-hydroxybenzoic acid did not inhibit incorporation, while acetylsalicylic acid showed a lower degree of inhibition. *In vivo* experiments on S^{35}-sulphate incorporation in rib cartilage of adult rats [5] showed essentially the same results.

The biochemical mechanisms underlying the inhibition of mucopolysaccharide synthesis by salicylates and other antiphlogistics in mesenchymal tissues is not yet completely understood. Various possible mechanisms have been discussed in studies by other authors [3 12 15] and in earlier papers from this laboratory [23, 24] One is depression of oxidative phosphorylation, causing a decreased production of adenosine triphosphate (ATP)

TABLE 3 *Incidence of rib anomalies and vertebral anomalies in alizarin-stained embryos from mothers given 10 mg of one of the substances i.m. on the 9th gestation day and in embryos from the untreated control group*

		Skeletal anomalies	
		Rib anomalies	Vertebral anomalies
COONa, OH (salicylate)	A/Jax	52.2 % of *03 investigated	33.3 % of *03 investigated
	CBA	16.7 % of 173 investigated	4.0 % of 1*3 investigated
COONa, OH (p-hydroxybenzoate)	A/Jax	0.0 % of 73 investigated	1.4 % of 73 investigated
	CBA	—	—
COONa, OCOCH (acetylsalicylate)	A/Jax	0.0 % of 61 investigated	1.6 % of 61 investigated
	CBA	3.1 % of 130 investigated	.3 % of 130 investigated
Control group	A/Jax	1.4 % of *09 investigated	1.0 % of *09 investigated
	CBA	0.0 % of 98 investigated	0.0 % of 98 investigated

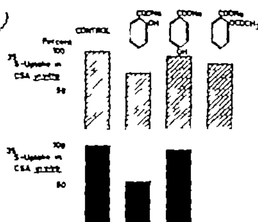


Fig. 1 Diagram showing the S^{35}-sulphate uptake in chondroitinsulphuric acid (CSA) in vitro and in vivo under the influence of sodium salt of salicylic acid, p-hydroxybenzoic acid and acetylsalicylic acid based on earlier investigations [5, 8]

and, consequently, of "active sulphate" (PAPS) which would then not be available for the sulphurylation of a mucopolysaccharide precursor. Recent data also point to an inhibition by salicylate of the enzyme L-glutamine-D-fructose-6-phosphate amino transferase which synthesizes glucosamine-6-phosphate, a key intermediate of mucopolysaccharide synthesis. Moreover, both in bovine heart valves [13] and in fetal tissues of mice [14] a similar difference was observed between salicylic acid and p-hydroxybenzoic acid with respect to potency as inhibitors of this enzyme as in the above mentioned in vivo and in vitro studies of mucopolysaccharide synthesis [5, 6].

The teratogenic effect as judged by the three parameters used, i.e. resorption of

embryos, vessel and skeletal anomalies, is pronounced for salicylic acid, which is also the most effective of the compounds depressing the synthesis of acid mucopolysaccharides.

The difference between the A/Jax and and CBA strains as regards susceptibility to the teratogenic action of salicylic acid seems, in fact, to be in agreement with the difference between the incidence of cortisone-induced cleft palate in the two strains [16].

Since nothing essential is known about possible differences in the metabolic fate of these teratogenic drugs in the two mice strains, no explanation can be given of the marked difference in teratogenic susceptibility. Incomplete understanding of drug metabolism also seems to be the main difficulty in interpretation of the results of teratologic experiments in various species and their applicability to man.

Summary

A/Jax and CBA primiparous mice were given a single injection of the sodium salt of salicylic acid, p-hydroxybenzoic acid and acetylsalicylic acid by the intramuscular route on the 9th or 12th day of gestation. The incidence of resorption and of vessel and skeletal malformations induced in the fetuses was estimated on the 18th day of pregnancy and compared with the spontaneous incidence of fetal malformations in a comparable control group. A significant increase in the incidence of fetal damage was obtained in the A/Jax strain with salicylic acid but not with the other two drugs tested. The correlation between these results and available data on the action of the three drugs on mucopolysaccharide synthesis is discussed.

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(H B), Wenner-Gren Institut
Norrtullsgatan 16
Stockholm
Sweden

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From the Departments of Pediatrics and Pediatric Radiology Kronprinsessan Lovisas Barnsjukhus and Karolinska sjukhuset Stockholm

Chronic Vitamin A Intoxication during the First Half Year of Life

Description of 5 Cases

by BENGT PERSSON RAGNAR TUNELL and KRISTINA EKENGREN

Since Joseph (1944) [11] described the first case of chronic vitamin A intoxication in children, more than 50 such cases have so far been reported. Almost without exception these cases have been children aged 1–5 years. The clinical picture has been uniform, skeletal symptoms in the terms of leg pain, swelling and roentgenographically verified cortical hyperostoses, signs of increasing intracranial pressure and skin lesions. The literature contains numerous surveys, the latest of these is by Oliver (1958) [19] The doses administered have been high, 80,000–500 000 IU of vitamin A per day often for more than 6 months. It has also been claimed that a characteristic feature of chronic vitamin A intoxication is its appearance after one year of age [4]. However in three cases previously reported the symptoms were observed earlier and at 6 months of age [1 16 27] (see Table 1) Since at this age the symptomatology differs from that in older children, and since correct diagnosis may therefore be difficult, we have made a survey of five cases, observed in Sweden during recent years, in which the ages were between 3 and 5 months. The toxic doses were small (18,000–60 000 IU of vitamin A) as compared with the doses previously reported, and the period of overdosing was short only one or two months.

Cases 1 2 and 4 have been briefly referred to earlier [23]

Description

Some descriptive data are presented in Table I. In all the cases pregnancy delivery and the neonatal period were normal, except for Case 2, where birth took place 4 weeks before term. All the infants had been fed adequately with breast milk or formula and when they were a few months old, juices and purés were added at one of the meals All the subjects had a normal psychomotor and somatic development.

Case 1

A boy who, following a diagnosis of craniotabes, had been prescribed a daily dose of 30 drops of vitamin AD[1] (22,500 IU of vitamin A) by his physician. The boy was given this dose when he was between 2½ and 4½ months of age. When he was 4 months old bulging fontanels were observed. At the age of 4½ months, anorexia and hyperirritability supervened and, consequently he was

[1] AD-vimin Astra, aqueous suspension. Ten drops correspond to 7500 IU of vitamin A and 1500 IU of vitamin D

TABLE 1 *Some observations on five cases of hypervitaminosis A and compared with three cases reported earlier [1 10 27].*

	J Arena et al 1951	J Nax t al., 1952	K. Wood and t al 1961	Case No. 1		3	4	5
Weight at birth, g	3,500	3,510	4 000	3,030	_590	_850	4 070	_[illegible]
Weight on admission t hospital, g	7,500			6,340	5,600	5 600	8,4[illegible]	6,4[illegible]
Age on admission t hospital, months	6½	9	2	4½	4	3	5½	4½
Vitamin A overdosages								
Dose IU itamin A/day	30,000→60,000ᵃ	*00,000ᵃ	70 000ᵇ	22,500	*2,500	60,000	*2,500	18,[illegible]
Age when overdosing began, months	0→4	½	0	½	3	1	3	1
Age t onset of intoxication symptoms, months	6	6	2	4	4	2½	5½	4
Clinical symptoms								
Anorexia and hyper irritability	+	+	+	+	+	+	+	–
Edema of the occipital area	–	+	+	+	+	+	+	–
Pronounced craniotabes	+	–	+	+	+	+	+	
Increased intracranial pressure	–	+	+	+	+	+	+	
Skin lesions, skin desquamation	–	+	+	+	–	+	+	
Laboratory findings								
Vitamin A fasting value IU/100 ml plasma (normal 40–100)	8.0	1 1 1	500	789	585	–	410	4 0
X ray findings								
Epiphyseal line changes	–	+	–	+	+	+		
Cortical hyperostosis	+	+	+	+	–	–	–	–

Oil solution. ᵇ Aqueous solution. Falling out of hair

hospitalized. H had tender edema at the back of his head,— tense bulging fontanels, separated widened sut res, "sun set" gaze circumference of head 44 cm (upper normal limit 40 cm) normal eyegrounds, and very pronounced craniotabes. On the palms and soles there was lamellar desquamation In addition he had rl ino-pharyngitis and bilateral otitis. The liver and spleen were not palpably enlarged.

During the next two week the boy wa gi en 10 drops of AD vimin[8] (500 IU f vitamin A) after which all tra tamin A was withheld During the first two weeks body weight decreased by 200 g the edema over the occipital region disappeared as did the sign of hydrocephalus, nd tl circum ference of the head diminished to 4 cm.

After a stay of 4 weeks in the hospital the boy was discharged as being entirely free of symptoms. At the follow up xamina tion when he was 3½ years of age he wa found to be healthy and normally dev loped.

Laboratory findings

The result of the plasma vitamin A deter mination are hown in T ble Tot l l pid 502 mg%, cholesterol *34 mg% Electropho-



Fig. 1 (Case 1) *Left* ( ) At the age of 4½ months the bones of the skull are very thin with relative sclerosis of the suture margins and widened sutures. *Right* (*b*) 1½ months later the calvarium has normal thickness and the sutures are normal.

reals on admission was normal with 6.6 g% of albumin. Hb 8.3–9.7g%. The WBC and the differential count were normal. Electrolytes in serum showed normal values for *potassium, sodium, chlorides, calcium and* phosphorous. Subdural and lumbar punctures were performed 2 weeks after admission. Subdurally 5 ml of liquor were obtained with the same albumin content, 36 mg%, as that following lumbar puncture. There was no increase in the number of cells. The alkaline phosphatases were elevated, 46–65–69 Bosch and Busch units (normally below 30 units at this age). The EEG was normal.

At 3½ years of age the laboratory findings were: Fasting vitamin A value in plasma 35 IU/100 ml of serum, alkaline phosphatase 18 Busch and Busch units.

Results of X-ray findings

At the age of 4½ months there was general reduction in the calcium content of the skeletal part investigated. The bones of the cranium were thin with relative sclerosis of the suture margins (Fig 1*a*). All the sutures were widened. Swelling of the soft parts was observed in the occipital area, but there was no periosteal reaction in the underlying bone. *In the wrists and ankles there were* changes in the metaphyses with widening and slight cupping the boundary with the epiphyseal cartilage was distinctly visible The changes were most marked in the ulna. In addition to this, there were small transverse decalcification zones proximal to the metaphyses. There were no changes in the humerus, femur proximal parts of the tibia, fibula or in the skeleton of the foot At 5 months of age the status remained unchanged. At 6 months of age a normalization of the bones of the skull had taken place (Fig 1*b*) The sutures were normal in width. Restitution had occurred in the wrist and ankles with regeneration of the transverse decalcification zones. In the lower tibia thin, sub-periosteal thickening had appeared. The calcium content of the skeleton had increased

TABLE 1 *Some observations on five cases of hypervitaminosis A and compared with three cases reported earlier [1 16 27]*

	J Arena et al 1931	J Nax et al., 195	K. Wood and et al., 1961	Case No. 1		3	4	5
Weight at birth, g	3,500	3,510	4 000	3,020	_500	2,850	1,870	2,9
Weight on admission to hospital, g	7,500			6,340	5,590	5,600	8 420	6,460
Age on admission to hospital, months	6½	9		4½	4	3	5½	4½
Vitamin A overdosage								
Dose IU vitamin A/day	30,000→80,000[a]	200,000[a]	70,000[b]	22,500	22,500	60,000	22,500	18,500
Age when overdosing began, months	0→4	½	0	2½	3	1	2	1
Age at onset of intoxication symptoms, months	6	6		4	4	2½	5½	4
Clinical symptoms								
Anorexia and hyperirritability	+	+	+	+	+	+	+	
Edema of the occipital area	−	+	+	+	+	+	+	−
Pronounced craniotabes	+	−	+	+	+	+	+	
Increased intracranial pressure	−	+	+	+	+	+	+	
Skin lesions, skin desquamation	−	+	+	+	−	+	+	−
Laboratory findings								
Vitamin A fasting value IU/100 ml plasma (normal 40–180)	850	1 121	500	769	885	−	410	479
X-ray findings								
Epiphyseal line changes	−	+	−		+	+	+	
Cortical hyperostosis	+	+	+	+	−	−	−	−

Oil solution. [b] Aqueous solution. Falling out of hair

hospitalized H had tender edema at the back of hi head,— tense bulging fontanels, separated widened sut ires, sun set" gare circumf rence of head 44 cm (pper normal limit 40 cm) normal eyegrounds, and ery pronounced craniot bes. On the palms and soles there was lamellar desquamation. In addition, he had hino-pharyngitis and bilateral otitis. The l ver and spleen were not palpably enlarged

During the next two week the boy was given 10 drop of AD-vitam [8] (500 IU of vitamin A) after which ll extra vitamin A wa w thheld. During the first two week body weight decreased by 300 g, the edema over the occipital region disappeared as did the signs of hydrocephalus, and the circumference of the head diminished to 4 cm.

After a stay of 4 weeks in the hosp tal, the boy was discharged as being entirely free of symptoms. At the follow-up xamination when he was 3½ years of age he w found to be healthy and normally developed

Laboratory findings

The result of the plasma vitami A determinations are hown in T ble 2. T tal l pid 50* mg* holesterol 231 mg*. Electropho-

value) 110 IU/100 ml of plasma, alkaline phosphatases 28 Busch and Busch units.

X ray findings

When the patient was 4 months old, the bones of the skull were thin and ppeared to be decalcified, and the suture margins were relatively sclerotic. There was moderat springing of the sutures, and occipital edema. In the wrists and ankles there was a slight widening and sharpening of the metaphyseal ends. There was no definit decalcification in the forearms and lower legs. At the age of 5½ months small clawlike exaggeration of the metaphyseal ends had developed distally in the femur and proximally in the tibia. Three weeks later there was a progress of the changes in the ulna with widening and cupping of the metaphyses. The changes in the tibia and the femur had progressed somewhat, but no lesions were bserved in the skeleton of the hands and feet When he was a little more than 7 months restitution had taken place. The skull was complet ly normal. At f llow up investigation t ages 2 and 5 years the skeleton of the arms and legs had an entirely normal ppearance.

Case 3

A girl for whom a daily dose f 20 drops of A D-ol aquosum [1] (60,000 IU vitamin A) wa prescribed at the Child Welfare Centre from the age of 3 weeks. At the age of 2½ months she developed over a period of 3 days, anorexia, hyperirritability and tender edema in the occipital region, and was therefore hospitalized. Increased fon tanel tension, pronounced craniot bes, ten der edema over the occipital region and general skin desquamation were observed. The liver and spleen were not palpably en larged.

It was immediately suspected that she was suffering from chronic vitamin A intoxica tion, and all supplementary vitamin wa withheld During the next 2 weeks the girl lost 300 g in weight, the fontanel tension normalized the edema disappeared, her appetit became normal, and after 3 weeks care she was discharged.

Laboratory find ngs

A determination of the fasting blood level of vitamin A proved unsuccessful Total albumin 6.0 g% with normal electrophoresis, serum calcium and phosphorus were normal Hb 7 g% alkaline phosphatases 29–30 Bodansky units (normal below 16 units)

X-ray f ndings

When th girl was 3 months old, the bones of the skull were thin with distinct sclerosis in the margins of the sutura sagittalis. In the skeleton of the wrists and ankles the metaphyses were somewhat widened, with levated margins, but with a sharp contour separating them from the epiphyseal carti lage The changes were most pronounced in the ulna. There was probably a slight general reduction of calcium in the skeleton, but no periosteal thickening

Case 4

A boy who between the ages of 3 and 5½ months, received bout 30 drops of AD vimin [b] daily (22,500 IU of vitamin A). H wa given this overdose because hi mother administered the full content of the dosage pipett without controlling the num ber of drops At the age of 5½ months he developed, during the course of three days, increasing irritability anorexia and tender edema in the occipital region, and wa consequently hospitalized On admission the fontanels were tense there was pronounced craniotabes, and skin desquamation on the palms and soles. The liver and spleen were not palpably enlarged. The eyegrounds were normal. The circumference of the skull was 47.5 cm (upper normal limit 46 cm).

During his stay in hospital no supplemen tary vitamin A was given, and he became free of symptoms during the foll wing weeks. An infection of the urinary passage was treated with tetracycline The boy was d s-

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A D-ol aquosum[a] Ferrosan, aqueous dispersion. 10 drops correspond to 30 000 IU of vitamin A and 4800 IU of vitamin D

TABLE 2 *Fasting values of vitamin A in plasma*

Case No.	Time after admission to hospital	Days after withdrawal of all *supplementary* vitamin A	Vitamin A IU/100 ml plasma
1	1 day	7	50
	4 da	10	210
	60 d	45	222
	1½ ears		585
	1½ years	78	460
4	78 days	78	410
5	6 d s	6	4 0
	45 da s	43	165

The method used for the plasma vitamin A determinations is described in a following paper [1]. Normal, f sting vitamin A level for hildren who received a prophylactic dose of 2500 and 7500 IU itamin A/day in aqueou solution, each respectively 67 ± 8 IU (= 19) and 95 ± IU/100 ml plasma (= 20) [1]

charged after four weeks with sligl t craniotabes.

The follow up examination when he was $3\frac{8}{12}$ years old showed that he was healthy and normally developed.

Laboratory findings

For plasma vit min A letermination see T ble 2.

The blood lipid on admission and when he was 9 month old were respectively cholesterol 308, 156 mg%, phospholipid 207 217 mg% (normal) nd for triglycerides 2.3, 1 18 mMol/l (normal not over 1 5 mMol l). Gas hromatographic investigation of the serum fatty-acid pattern wa normal H parent and later had normal blood lipid values. Tot l albumin 5 6 g% w th normal electrophoresis. Hb 1—— g% to 9 6 g% The WBC and the differential count were normal. The bleeding and coagulation times were normal. Serum lectrolytes, potassium, sodium chlorides, calcium and phosphorus normal. The alkaline phosphatases were normal, 13 Busch and Busch unit Protein



Fig (Case 4) At 6 months of age widening of the metaphyses in the wrist The margin are sharpened The ulna met physis l cupped. Not distinct margin of epiphyses.

bound iodine 6.5 % BMR − 14 %. Normal amino-acid pattern in the urine Lumbar puncture on admission howed normal number of cell and an albumin content 4 41 mg%

At the age of $3\frac{8}{12}$ years, the fast ng value for vitamin A was 100 IU 100 ml plasma.

X ray findings

When the patient w hardly months old, the bones of the skull were rather thin with sclerotic suture margin d moderat springing of sutures. There w occipital edema withou t periosteal reaction in the underlying bone In the wrist and ankles the metapl yses were widened nd cupped, and with a sharply def ned mar nal zone separating them from the epiph seal cart lage There was no definit decalcification in the keleton. The hanges were most marked in the ulna nd fibula (Fi). At 6½ month of age the condition of the wrist and ankles w unchanged. There were lecalcification zones proximal t he met physes In the phalanges of the hand there wa slight w lening and tensifie tion of the metaphyseal ends. At 7½ montl of age the kull sut res were of normal w dth nd the wrist and ankles of normal ppearance Skelet l film at age $3\frac{8}{12}$ ears were normal

Case 5

A girl for whom a daily dose of 4 drops of AD-vimin® (17,500 IU of vitamin A) was prescribed at the Child Welfare Center from the age of one month. When she was between 4 and 4½ months old her mother observed slight bulging of the fontanels. Over a period f 24 hours the bulging of the fontanels became more obvious and the hild was pathetic to some extent, and was consequently hospitalized On admission it was found that the fontanels bulged slightly more than ½ cm bove the plane of the skull. The circumference of the skull was 4.. cm (upper normal limit 43 cm). She had craniot bes but no swelling of the soft parts and the skin was normal. There was no palpable enlargement f the liver or spleen. The eyegrounds were normal.

It was immediately suspected that she wa suffering from hypervitaminosis A, and all supplementary vitamin A was withheld. The fontanel tension was normalized in a few days, and the patient craniotabes had completely disappeared after on month. She was discharged as healthy after two weeks.

Laboratory findings

For plasma vitamin A determination, see T ble 2. The fasting values for the blood lipids were on admission, and one month later respectively: cholesterol 140 and 122 mg% phospholipids 160 and 146 mg% triglycerids 2.23 and 0.65 mM l/l (normal not over 1.5 mMol/l) Total albumin 5.9 g%, normal electrophoresis. Hb 10.1–12.7 g%. The WBC and the differential count were normal. The serum lectrolytes, potassium, sodium, chlorides, calcium and phosphorus were normal. The alkaline phosphatases were normal 10–14–16 Bosch and Busch units. Lumbar puncture performed the day after admission showed no cell increase and the albumin content was normal, 19 mg% The urine was found t contain no albumin glucose or increased amount of amino acids.

X-ray findings

When the patient was hardly 5 months old the cranial sutures were somewhat widened. In the wrist and ankles the meta physes were widened, sharply demarcated. The changes were most pronounced in the ulna. Proximal to the metaphyses, decalcification zones were indicated (Fig 3) There was no definit general decalcification. At the age of 5½ months the status was un changed At the age of 6 months there was regression of the changes, but the meta physes of the ulna were still widened and cupped When the girl was 9 months old, conditions in the wrists and ankles were normal.

Discussion

Table 1 gives a survey of cases of hyper vitaminosis A, before and at the age of 6 months previously reported in the literature The symptomatology of these 3 cases is in close agreement with that of our 5 cases, which may be regarded as representing different degrees of severity in chronic vitamin A poisoning Signs of increased intracranial pressure were evident in all our cases, nd can be regarded —as earlier pointed out by Rothman & Knudson—as a "connecting link" between the acute and the chronic forms of vitamin A intoxication [21]. The history of in creased vitamin A intake for a prolonged period, the occurrence of an elevated fast ing, blood vitamin A level, nd the demonstration of roentgenological skeletal changes represent the essential diagnostic criteria for chronic intoxication.

The typical skeletal changes in intoxi cated infants older than 6 months of age are cortical hyperostosis on the tibia fibula clavicle and met tarsal bones and tender swelling of the soft tissue covering

TABLE 2 *Fasting values of vitamin A in plasma*

Case No.	Time after admission to hospital	Days after withdrawal of all supplementary vitamin A	Vitamin A IU/100 ml plasma
1	1 days	7	00
	4 days	16	210
	60 d	45	[illegible]
2	1½ years		535
	1¾ years	36	66[illegible]
4	5 da	78	410
5	6 days	6	4 0
	43 da	45	143

The method used for the plasma vitamin A determination is described in following paper [1] Normal fasting vitamin A level for children who received a prophylactic dose of 500 and 500 IU vitamin A/day in aqueous solution, each respectively 67 ± 8 IU (19) and 95 ± IU 100 ml plasma (= 9) [4].

charged after four weeks with slight craniotabes

The follow up examination when he was 3_{12} years old showed that he was healthy and normally developed.

Laboratory findings

For plasma vitamin A determination see Table

The blood lipids on admission, and when he was 9 months old were respectively cholesterol 208 158 mg% phospholipids 0" 17 mg% (normal) and for triglycerides 3. 1 16 mMol l (normal not over 1 8 mMol l) Gas chromatographic investigation of the serum fatty-acid pattern was normal. His parents and sister had normal blood lipid values. Total albumin 5 6 g% with normal electrophoresis Hb 1 g% to 9 6 g% The WBC and the differential count were normal. The bleeding and coagulation times were normal. Serum electrolytes potassium, sodium, chlorides, calcium and phosphorus normal. The alkaline phosphatases were normal 12 Bosch and Busch unit Protein-



Fig. — (Case 4) At 6 months of age widening of the metaphyses in the wrist. The margins are sharpened. The ulnar metaphysis is cupped. Note distinct margin of epiphyses.

bound iodine 6.5 γ% BMR 14 %. Normal amino-acid pattern in the urine. Lumbar puncture on admission showed normal number of cells and an albumin content of 41 mg

At the age of 3_{12} years the fasting value for vitamin A was 100 IU 100 ml plasma.

X-ray findings

When the patient was hardly six months old, the bones of the skull were rather thin with sclerotic suture margins and moderate springing of sutures. There was occipital edema without periosteal reaction in the underlying bone. In the wrist and ankles the metaphyses were widened and cupped, and with a sharply defined marginal zone separating them from the epiphyseal cartilage. There was no definite decalcification in the skeleton. The changes were most marked in the ulna and fibula (Fig.). At 6½ months of age the condition of the wrists and ankles was unchanged. There was decalcification ones proximal to the metaphyses. In the phalanges of the hand there was slight widening and otrosclerosis of the metaphyseal end. At months of age the skull sutures were of normal width and the wrist and ankles of normal appearance. Skeletal films at age 3_{12} years were normal

animals with induced hypervitaminosis A. [17] Hyperostoses do not occur but a pathological brittleness of the bones is observed resulting in spontaneous fractures. Thomas *et al* [22] have shown in the rabbit that as early as 3 days after over dosing has begun a disturbance in mucopolysaccharide biosynthesis occurs, with a liberation of chondromucoprotein from the cartilaginous matrix of the epiphyseal plates, which is reflected in a rise of chondroitin sulfate in the blood. The final result is, as has also been demonstrated in the guinea pig and the rat, a premature closing of the epiphyses [?6]

Further evidence concerning the mode of action of vitamin A has accumulated during recent years. The changes observed in cartilage exposed to an excess of vitamin A are said to be caused by a changed stability of the cytoplasmic particles known as lysosomes. Dingle has shown in vitro as well as in vivo in hypervitaminotic rats that vitamin A alcohol has a direct effect on lysosomal particles and thus probably on the permeability of the surrounding lipoprotein membrane resulting in an increased liberation of lysosomal hydrolytic enzymes [5].

Recently Pease has described an additional condition following chronic vitamin A intoxication In three out of seven children—focal growth disturbances in the long tubular bones as a consequence of premature epiphyseal closure were reported [20] All these children were more than 1 year old (varying between 13 months and 3 years) when intoxication occurred, and in some cases in addition to cortical hyperostoses, they also displayed irregularities in the epiphyseal plates. The follow up investigations were made between 3½ and 14 years later The follow up investigations on our cases that were carried out between 3 and 4½ years after intoxication (Cases 1 ? and 4) showed no disturbances in the growth of the skeleton, the bone nuclei and the metaphyses had a normal X ray appearance. Very probably this is connected with the fact that these patients had not begun to overburden their skeletons at the time the intoxications took place

Besides spontaneous fractures in the diaphyses, Ehrengut has pointed out that in the rabbit even the flat bones in the skull are decalcified [9].

All the patients had pronounced craniotabes which, in the most severe cases, were associated with tender swellings of the soft parts in the occipital region. The X-ray examinations showed that in 4 out of the 5 children the bones in the skull were thin and decalcified. Seen against the background of the other skeletal changes, it seems probable that these in the skull are directly caused by hypervitaminosis and are not, as has been suggested, secondary to the increased intracranial pressure [7] In all cases the sutures were widened as a consequence of the increased intracranial pressure

The causes of the rise in intracranial pressure have been discussed at length. Increased production of cerebrospinal fluid, a reduced absorption of this fluid or a combination of the two [2] One can speculate if the increased intracranial pressure might be attributed to an alteration in the permeability of the blood—brain barrier—caused by an excess of vitamin A alcohol and compatible with it effect on lysosomal particles.

Apart from the determination of the

fasting value for vitamin A, little assistance in establishing a diagnosis of hypervitaminosis A is obtained from laboratory tests. However, in children the alkaline phosphatase activity is frequently increased whereas the calcium and phosphorous values are normal. Whether this is a reflection of changes in the liver or the skeleton is not clear. It has been demonstrated that in rats suffering from hypervitaminosis the epiphyses have an increased content of alkaline phosphatases as well as the interlobular structures of the liver [14, 15].

Both in hypervitaminosis A and in carotinemia an increase in the total lipids and cholesterol has been reported [11]. In rats with vitamin A deficiency C^{14} labeled mevalonic acid is incorporated more slowly into cholesterol than under normal conditions [25]. Whether the contrary condition holds good for hypervitaminosis is not known. No definitely elevated cholesterol values were noted in the cases investigated, but in two of them (that were investigated in this connection) there was transitory hypertriglyceridemia. A postulated antagonism between vitamin A and thyroxin has been given as an explanation of the rise in lipid level. Normal BMR and protein bound iodine in one of the cases, however, does not support hypothyroidism. In the same case gas chromatographic analysis of the blood fatty-acid pattern showed normal distribution which apparently excludes a possible connection between the large lamellar epidermal scaling and linoleic acid deficiency [10].

Hypertension has not been reported in infants with hypervitaminosis A. Thus of particular speculative interest is Case 2 in which a distinct vascular hypertension, decreased creatinine clearance and persistently elevated alkaline phosphatase levels prompted the initial diagnosis of hypertension of renal etiology, suprarenal and central nerves system causes having been excluded. In retrospect, however, it appears highly probable that these manifestations were secondary to hypervitaminosis A. A reduction of vitamin A below 7500 IU/day effected a complete remission over a 5-year period. There was no residual renal impairment. In hypervitaminotic rats lipid infiltration of the reticuloendothelial system as well as lipid deposit in the glomerular endothelial cell and renal cortex have been observed [6, 17, 18]. Reasonably then, one might suspect the hypertension of our case to be secondary to renal damage of similar metabolic etiology.

Infantile cortical hyperostosis is a differential diagnosis of hypervitaminosis A. Formerly it was asserted that the age at onset distinguished the two condition [4]. It is evident that both can start before the age of six months.

The accumulation of diagnosed cases of intoxication which have occurred in Sweden in recent years is worthy of note. Two further cases, where the patients were under 6 months of age, have been established at the Children's Hospital in Göteborg [8].

Up to 1955 the AD preparation mainly used in Sweden were oil solution representing a daily prophylactic dose of 2000-3000 IU of vitamin A. After the introduction of vitamin AD soluble in water a gradual change has occurred in so far as these preparations are now being increasingly used. They are equivalent to a daily

prophylactic dose of 7500-10,000 IU of vitamin A. However in common with oil solutions the vitamin D dosage remains unchanged at 1500 IU As early as 194 Lewi was able to show in infants, that an aqueous dispersion of vitamin A in single doses of 1.,500 or 35,000 IU gave in comparison with an oil preparation, a blood concentration that was about 4 times higher and a smaller fecal loss of vitamin [13]. In all our cases intoxication was caused by vitamin AD in aqueous dispersion. A therapeutic vitamin D dose of 4500 IU of vitamin D represents in these preparations, a vitamin A dose of 22,500 IU per day which, in our cases, was shown to be a toxic dose Consequently it is not remarkable that in four of these cases the increased vitamin AD doses were prescribed by a physician, and in this respect they differ from e.g Oliver s *survey of chronic hypervitaminosis*, where only out of *7 cases received an overdose owing to a physician s prescription [10]. The A-dose in the Swedish preparations consisting of aqueous solutions, is remarkably high in comparison with the internationally used and recommended dosage of 1500-*000 IU/day and therefore a reduction to a corresponding level is proposed.

Summary

Chronic hypervitaminosis A is described in five children whose ages range from 3 to 5½ months. The periods of overdosing were short varying between 1 and 3 months and the doses (18,500-60 000 IU vitamin A/day) were considerably lower in comparison with those of the cases reported earlier The reason for this is, that all the patients received vitamins in readily absorbable aqueous dispersions. A reduction of the prophylactic vitamin A dose, in the Swedish water-soluble AD-preparations from 7500 to 2500 IU A/day is suggested The characteristic features of the cases of intoxication are in addition to the elevated fasting value of vitamin A in plasma, anorexia, irritability increased intracranial pressure skin desquamation, occipital edema and pronounced craniotabes. The roentgenological findings were in the most advanced cases a general reduction of the calcium content in the skeleton. In all the cases, cup-shaped deformations of the widened metaphyses were observed, which were sharply demarcated from the epiphyseal cartilages in the skeleton of wrists and ankles. Only in one case did there appear in the course of the disease a periosteal thickening in the distal tibia—without swelling in the soft parts. The withdrawal of vitamin A led to cure in all the cases; and follow up *investigations of three of these patients* 2-4½ years after intoxication, showed normal skeletal growth.

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(B. P.), Department of Pediatrics
Kronprinsessan Lovisas Barnsjukhus
Polhemsgatan 30
Stockholm K
Sweden

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From the Departments of Pediatrics I Karolinska sjukhuset and Kronprinsessan Lovisas Barnsjukhus and the Department of Clinical Chemistry of Beckombergs sjukhus, Stockholm

Prophylactic Vitamin A Dose in Sweden

An Investigation in Connection with Cases of Intoxication

by RAGNAR TUNELL, LARS-GÖRAN ALLGÉN BIRGITTA JALLING and BENGT PERSSON

Because of the 5 reported cases of chronic vitamin A poisoning among infants aged 3–5 months, which have occurred in Sweden in recent years [14], the question of a suitable prophylactic vitamin A dose has become of immediate interest The investigations on which the assessment of the optimum vitamin A administration to infants is based, were concerned with the lower limit of vitamin A needs, i.e. the dose required to prevent the occurrence of deficiency symptoms [6 8]. These investigations showed that the administration of 300 IU of vitamin A per day to infants may mean a possible risk of hypovitaminosis A with changes in the vaginal epithelium [6] and a lowered blood vitamin A level [8]. The dose of 1500 IU of vitamin A per day to infants, which is recommended by the National Research Council in the U.S.A. [15] is intended to ensure an adequate safety margin against the occurrence of hypovitaminosis A. No investigations have been reported to determine the upper limit of the prophylactic dose i.e against the risk of hypervitaminosis A.

During the last 10 years in Sweden, vitamin AD preparations have been used to an increasing extent where the recommended prophylactic dose per day contained 7500–10 000 IU of vitamin A and 1500 IU of vitamin D The vitamins in these preparations are dispersed in colloidal aqueous solution, which allows more ready absorption than does oil solution [5 7]. It has been shown that the daily administration of a vitamin A dose which is 2–3 times higher can be toxic [14] Consequently it was considered of interest to investigate whether a daily dose of 7500 IU of vitamin A in aqueous solution, administered to infants up to 3–5 months of age could involve a risk of hypervitaminosis A, and this dose was administered to a group of premature and mature infants. As a control, another group was investigated which received the recommended prophylactic dose of 2500 IU of vitamin A, which is in general use internationally A third group was given a single dose of 22,500 IU of vitamin A, it had been found that this dose could prove toxic in long-term treatment

TABLE 1 *Results of some serum analyses in premature infants 3–5 months after birth on a prophylactic dosage of vitamin A at 2500 and 7500 IU/day*

Case no.	Vit. A dosage IU/day	Serum calcium mg %	Serum inorganic phosphat mg %	Serum alk. phosphatase Bosch & Bosch units	Serum protein g %
13	2500	10.6	6.6	18	5.6
14	2500	10.9	5.9	27	5.5
15	2500	8.4	7.0	30	4.1
16	2500	10.9	10.9	38	5.5
17	2500	10.[illegible]	6.4	5	6.0
18	2500	9.9	9.0	26	5.2
19	2500	10.7	[illegible].0	38	5.5
Mean values		10.[illegible]	6.0	29	5.5
38	7500	11.5	10.6	24	[illegible]
39	7500	9.7	—	—	—
41	7500	11.0	8.9	27	5.8
42	7500	8.8	6.5	20	5.1
43	2500	10.[illegible]	5.5	29	4.6
44	7500	—	9.9	23	5.8
45	7500	10.4	6.6	23	6.0
46	7500	9.1	5.8	—	—
47	7500	11.2	9.0	26	6.[illegible]
48	2500	10.2	5.6	32	[illegible]
49	7500	10.2	5.6	—	5.0
Mean values		10.[illegible]	6.2	26	5.4

Material and Methods

The "double blind" technique was used in the investigations into the vitamin A doses of 7500 and 2500 IU. A numbered series of bottles of vitamin AD (kindly supplied by AB Astra) containing a daily dose (10 drops) of 1600 IU of vitamin D and 7500 or 2500 IU of vitamin A was used.

From the age of one week, the preparation was administered to twenty prematurely born infants. They were readmitted when they were from 3 to 5 months old, clinical examinations were then carried out with special reference to any possible symptoms of hypervitaminosis A (hyperirritability, signs of increased intracranial pressure, occipital edema, lamellar scaling) and to the determination of calcium, inorganic phosphate, alkaline phosphatase and total serum protein as well as to the X ray examination of the wrists and ankles. The plasma vitamin A level, both as a "fasting value", i.e. about 1[illegible] hours after the preceding meal, and about 20 hours after the preceding daily dose of vitamin A, and as a "four-hour value", i.e. 4 hours after the administration of the prophylactic dose previously used per day given with a meal was determined.

From the age of 2 weeks the preparation was administered to forty mature infants. Twenty five of them were subjected to out-patient follow-up examinations when they were 3–5 months old, at which time "fasting values" and "four hour values" for vitamin A were determined.

Six children aged 3–5 months and with

The preparations consisted of AD-vitamin® containing vitamin A 25,000 IU/ml, calciferol 5000 IU/ml, sorbimacrogol oleas 300 mg/ml, preservative pH-corrective, and of AD vitamin ® containing 6800 IU ml of vitamin A, otherwise identical with AD-vitamin ®

previous histories of varying vitamin A prophylaxis and feeding (Table 1) were given a single dose f 22,500 IU of vitamin A, as 30 drops of AD-vimin[1] The fasting value four-hour and eight hour values of vitamin A in plasma were determined.

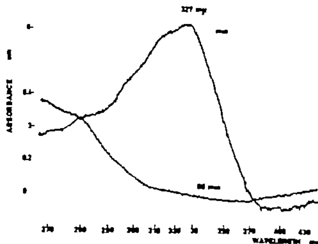
The blood samples—5 ml in heparinized tubes—were drawn from one internal jugu lar vein. Subsequently plasma was separated. If stored, plasma was kept in the dark.

The vitamin A determinations were carried out by the U V spectrophotometric method of Paterson & Wiggins [13].

In a horizontal shaking machine (Buhler) 0.75 ml heparinized plasma, 1.5 ml absolut ethanol and 1 5 ml n-heptane were shaken for 15 minutes. The layers were then allowed to separat and the heptane layer was transferred t a sodaglass tube (1 mm wall thickness) with glass stopper Part of this sol tion was filled in a semimikro silica cell with cover (10 mm optical path, approx. 1 ml volume) and the absorbance at 327 mμ was determined in a Zeiss PMQ-II spec trophotometer Heptane was used for the blank setting

After this measurement the heptane solution was returned to the glass tube with glass stopper and was irradiated for 90 minutes with longwave U V light (Luma Hg U 1*0 W lamp with Wood glass bulb)





b

Fig 1 (a) Absorbance curves for vitamin A acetate (IUPAC ref. stand.) in heptane solution (18 3 IU/ml) before and after arious irradiation times. (b) Absorbance curves for a plasma itamin A extract before and after irradiation for 90 minutes. Calculated vitamin A content was 1225 IU/100 ml plasma.

in a standardized geometrical arrangement

The time for irradiation was tested and some results are given in Fig 1 *a* and *b* for standard and plasma extract.

The absorbance after irradiation was then measured, from the two values before and after irradiation thus obtained for each sample the difference was calculated

$$\Delta A(327\ m\mu/1\ cm)$$

The method was standardized with heptane solutions of IUPAC vitamin A reference standard (crystalline vitamin A acetate in vegetable oil) ΔA(327 mμ/1 cm) for 1 I U vitamin A/ml heptane was then found to be 0 046 under the conditions used by us.

$$\Delta A \frac{327\ m\mu}{1\ cm} \cdot \frac{1}{0.046} \cdot 100 = \Delta A \frac{327\ m\mu}{1\ cm}$$

$$4350 = \text{I.U vitamin A/100 ml plasma.}$$

This holds good if the yield on extraction is 100%. This was not tested by Paterson & Wiggins but Ollendorff states a loss of around 40% on extraction [12] The yield is not easy to check because plasma with known additions of vitamin A must be used and the vitamin should be in the same physical and chemical stat as in normal plasma W used the following procedure. Arovit® (Roche) which is vitamin A palmitate brought into aqueous solution by the use of a solubilizer was first diluted with absolute ethanol (1 10) and then with heptane for direct analysis of vitamin A content or with water and serum for extraction and analysis of vitamin A content We then obtained a yield varying between 81 and 93%, with a mean of approx. 87%. As we did not look upon this method of testing as completely satisfactory we have not introduced any correction for low extraction yield. Thus our analytical figures may be a little too low (pprox. 13%) The results are given as th mean values of double determinations. The error of method calculated on the basis of 92 double determinations was ± 14 IU

Results

In the entire material not one single case with signs of hypervitaminosis A was found, and the frequency of craniotabes was the same for children who received 2500 IU of vitamin A per day as for those who were given 7500 IU daily

The results of the serum analyses for protein, calcium, phosphate and alkaline phosphatases in the premature infants are given in Table 1 There was no demonstrable difference between the children who received 2500 IU and those who received 7500 IU of vitamin A per day The roentgenologic investigation of the hand and foot showed normal conditions in all of the cases except one (No. 49), in which subperiosteal thickening of the radius and the ulna without widening or cupping deformation of the metaphyseal ends occurred. Control X ray examinations carried out 2 months later showed normal conditions.

The results of vitamin A determinations in plasma, after a daily dose of 2500 or 7500 IU of vitamin A, are shown in Fig 2 Since it was not possible to establish a positive correlation between the children's weight at birth or weight at the time of the investigation and the vitamin A content of the blood, the results of the investigations for premature and mature infants are given together The mean fasting vitamin A content in plasma, following a daily administration of 7500 IU was 95 ± 8 IU/100 ml (n = 29) whereas after the administration of 2500 IU it was 68 ± 6 IU/100 ml (n = 19) i.e somewhat lower (significant on the 5% level) The corresponding "4-hour values were 334 ± 26 IU/100 ml and 140 ± 10 IU/100 ml respectively Thus, the vitamin A content

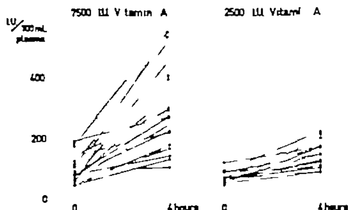


Fig. 2. Graphic illustration of the results of vitamin A determination in plasma after a daily dose of 7500 and 2500 IU of vitamin A in an aqueous preparation as supplement of vitamin A. The determination was made before and 4 hours after administration of the daily dose.

in plasma was definitely higher after administration of the larger dose (difference significant on the 0.1 % level).

The six children who received a single dose of 22,500 IU of vitamin A (Table) had, 4 hours after the administration of this dose, 300–750 IU of vitamin A/100 ml plasma. No symptoms of intoxication were observed.

Discussion

No symptoms of vitamin A intoxication occurred in the premature and mature infants, following the administration of vitamin A in a dose of 7500 IU per day in aqueous solution. This was hardly to be expected, since this dose had been used prophylactically for infants in Sweden for 10 years. In one case a premature infant (No 40), the X ray examination revealed periosteal thickening of the metaphyseal end of the radius and the ulna. Such changes are reported in infants with vitamin A intoxication [1]. However infants with chronic vitamin A intoxication, at the age of 3 to 5 months, display cupping

TABLE 2. *Results of vitamin A determinations in plasma from children receiving a single dose of 22,500 IU vitamin A*

Case no.	Sex	Birth weight g	Weight at examination g	Prophylactic dose of vit. A IU/day	Diet	Fasting value IU/100 ml plasma	4-hour value IU/100 ml plasma	8-hour value IU/100 ml plasma
W 000021	M	3640	6450	15,000	Artif.	79	333	—
G 000709	M	3760	7000	7,800	Mixed	87	376	707
D. 000625	M	2920	7900	2,500	Br milk	75	720	490
L. 000903	M	2330	7420	15,000	Br milk	144	413	—
D 000817	M	2870	4420	7,500	Mixed	130	—	1676
T 000406	F	2940	7060	,500	Mixed	265	736	—

TABLE 3 *Fasting plasma vitamin A values in infants at 3–5 months of age. Data in present study compared with those reported in the literature.*

Authors	Methods	Material			Plasma vitamin A IU/100 ml	
		Months of age	No. of cases	Supplements	Mean	Range
Lewis et al 1941 (5)	Photometry based on Price Carr reaction	3–6	11	No supplement of vitamin A	78	56–111
		3–6	22	Supplement of 17,000 IU in oil preparation per day	93	50–160
Kübler 1957 (6)	Photometry based on Glycerol dichlorhydrin reaction	3–5	20	No supplement of vitamin A	90	40–190
Present study	UV photometry and destruction of vitamin A by UV irradiation	3–5	20	Supplement of 2500 IU in aqueous preparation per day	84	50–111
		3–5	29	Supplement of 7500 IU in aqueous preparation der day	95	44–187

deformations and a widening of the metaphyseal ends [14]. No such changes were observed in this case The control examination conducted 2 months later showed that the X ray of the skeleton was normal despite the fact that the infant had received the same vitamin A dose of 7500 IU per day up to the time of control examination. The results of the vitamin A determinations in plasma (fasting value 44 and 4-hour value" 177 IU/100 ml) appear to refute the view that hypervitaminosis A was the cause of the skeletal changes and, consequently this is not regarded as a case of vitamin A intoxication. Table 3 shows the fasting values of vitamin A in infants aged 3–5 months, which were previously reported in the literature The fasting values found in this investigation after the administration of 2500 and 7500 IU of vitamin A, given as a prophylactic dose are in agreement with the results given in the literature In all the materials however there are wide individual variations and the values tend to be lower for these ages than for older children and adults where 131 IU/100 ml plasma is reported by Moore as a mean value for 1040 investigations [10]. The mechanisms for the regulation of the fasting vitamin A content in plasma are unknown [4] A value below 40 IU/100 ml is obtained only after vitamin A has been withheld for a long period [8], and a value of 400 IU/100 ml or more occurs only after prolonged overdose [11] However it is noteworthy that one of the children in the group who received a single dose of 22,500 IU of vitamin A, but who had previously been given a prophylactic dose of 500 IU of vitamin A per day had a fasting vitamin A value of 965 IU/100 ml, and that 3 of the immature infants who received 7500 IU of vitamin A per day had a corresponding plasma value of 170–190 IU/100 ml of plasma For both

this investigation and other published series as well these fasting values are remarkably high for infants who are less than 6 months of age

If the blood vitamin A content following a vitamin A dose is considered in relation to time a "vitamin absorption curve" is obtained. The area under this curve has been regarded as proportional to the amount of vitamin A absorbed from the intestinal canal [*]. Since after the administration of vitamin A in aqueous solution, the maximum of this absorption curve occurs at about 4 hours, a sample taken at this time can give a rough idea of the relative degree of absorption of vitamin A after different doses. After the administration of 22,500 IU given as a single dose an increase in the vitamin A content to between 300 and 750 IU/100 ml is obtained. After a dose of 7500 IU there was an average increase up to 334 IU/100 ml, one half of the cases had an increase to between 300 and 600 IU/100 ml, i e. the same order of magnitude as that following 22,500 IU All the children who were given the reduced dose of 2500 IU had a 4-hour value that was less than 220 IU/100 ml.

Thus, daily administration of 7500 IU aqueous vitamin A often increases the plasma levels of vitamin A remarkably Although not demonstrably toxic these increases may well represent the upper limit of vitamin A tolerance in sensitive individuals. On the other hand a daily dose of 2500 IU of vitamin A gives adequate security against the risks of overdosing This dose is also in closer agreement with the internationally applied [3] and recommended [9 15] prophylactic dose for infants

Summary

The administration of vitamin A to infants in an aqueos solution and in a dose of 7500 IU per day has been studied in view of the reported occurrence in Sweden of 5 cases of chronic vitamin A intoxication among infants aged 3–5 months. The fasting blood value and the 4-hour blood value of the vitamin A content have been determined for 54 infants aged 3–5 months following the administration of vitamin A in a dose of 22,500 IU 7500 IU or 2500 IU to three groups of infants. In comparison with 2500 IU of vitamin A, the administration of 7500 IU of vitamin A tends to produce higher fasting values. In four cases the fasting values are up to 900 IU which must be regarded as remarkably high for this age group. In the group which received 7500 IU of vitamin A, the vitamin A content after 4 hours was significantly higher than that for the infants who were given 2500 IU Half of the cases which received 7500 IU of vitamin A had 4-hour values of the same order of magnitude as that of the infants to whom a single dose of 22,500 IU had been administered. The latter dose, when given daily had been found to involve risk of chronic intoxication. Consequently the latent risk of hypervitaminosis A cannot be excluded with a daily dose of 7500 IU of vitamin A, even if it has so far been impossible to verify any case of chronic vitamin A poisoning when this dose is given. Thus we recommend that the vitamin A content in the Swedish preparations be red ced to a dose of about 2500 IU of vitamin A per day which also corresponds to the internationally recommended prophylactic dose of 1500–2500 IU per day

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(R T). Department of Pediatrics
Karolinska Sjukhuset
Stockholm 60
Sweden

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From the Children Department and Pediatric Research Institute University Hospital, Rikshospitalet, Oslo

Normal Red Blood Picture during the First Three Years of Life

by PETER JOHAN MOE

Introduction

The word normal is used in the sense of denoting the blood values of healthy infants and children [3 16]. Most cases of iron-deficiency anemia occur during the first three years of life, and it is important, therefore to establish especially the lower limits of normal hemoglobin concentration, hematocrit and red cell indices in this age period.

The literature dealing with the red blood picture in infancy especially hemoglobin concentration, is voluminous, and there is a wide range of values for normal standards recorded by different authors on this subject. The difficulties in finding comparative data may be summarized in the following points.

1 Inclusion of anemic infants in normal series
2 Differences in the standardization of the techniques
3. Too few observations at the different age levels
4 Inclusion of prematurely born infant
5. Lack of completeness of data

The importance of the first two points is demonstrated in Fig 1 The mean hemoglobin concentration in our studies has been compared with the curves of 5 large "normal series" from the literature There are only a few workers who have performed studies of infants receiving an adequate daily iron intake during infancy but it is obvious that the secondary fall in hemoglobin concentration can be altered and an increase obtained. Most standards used today for the normal red blood picture [2, 15 24] were laid down 20–30 years ago and cases of iron-deficiency anemia seem to have been included [1]

The studies by Mackay [13], Elvehjem *et al.* [6] and Nicum *et al.* [19] of infants receiving an adequate iron intake include only small numbers of infants in each age group hemoglobin determinations only were performed, and for other reasons too it is difficult to use them for comparison in this article. The close relationship between Sturgeon s and our figures for mean hemoglobin concentration at the age of 6 to 1 months has already been demonstrated [16]

An attempt will be made in this article to compare our figures with those of other Scandinavian workers. There are however no other studies from Scandinavia of full-term infants receiving an adequate daily iron intake and Vahl

quist [22] is the only one who gives figures for the complete normal red blood picture during infancy In his comprehensive studies of serum iron, there are relatively few observations on the normal red blood picture after the age of 1 month (whilst in our studies little attention has been paid to the neonatal period)

Material and Methods

The data in this article are taken mainly from the author's longitudinal studies of iron requirements during infancy [16 17]. A total of 10*0 blood specimens have been studied from 420 infants and children up to the age of 3 years. The blood values of all healthy infants up to the age of 2–3½ months have been considered normal regardless of dietary iron content (A high daily iron intake during the first months of life may possibly result in a small increase in mean hemoglobin concentration at the age of 3½ months. This however seems to be relatively unimportant as long as the infants reach normal hemoglobin concentrations at the age of 6–7 months [16].) After the age of 4 months we have included the blood values of all healthy infants and children participating in the previous studies, and receiving an adequate intake (groups A, B and \ —with exceptions for three cases which received supplementary iron at the age of 1–3 years [16 17]).

The blood studies were performed on capillary blood from the fingertip (or blood from the heel in the smallest infants), with the same technique as in clinical practice "Medi point" blood lancets have been used throughout in the studies. There is a difference between simultaneous blood studies in capillary and venous blood during the first days of life [*2] The use of a deep cut permitting free blood flow from the heel in neonates will also result in considerably lower blood values than in our studies. This, however is seldom done in clinical practice. Figures comparatively high as ours during the first days of life have also been found by other workers using capillary blood for hemoglobin and red blood cell determinations [7 18, 20], and the same high figures have been found in the Children's Hospital in Bergen (unpublished data) Both the oxyhemoglobin and cyanmethemoglobin methods were used in the hemoglobin determinations. Calibration curves were obtained using a recognized standard solution of cyanmethemoglobin and a Beckmann D U spectrophotometer The MM extinction coefficient was 11.4. There was a close relationship between the two methods, and the reported means were only about 0 05 g/100 ml below the means with the cyanmethemoglobin method at the age of to 12 months.

Hematocrit was determined in duplicate (except in the beginning of the studies) by the micromethod using a Ljungberg microhematocrit centrifuge The diameter of the centrifuge was 190 mm and its speed 8000 r p.m. The diameter of the microtubes was 0.8–1 0 mm, and their lengths 75 mm, the tubes contained dried heparin The speed of our centrifuge was sufficient to secure close to a constant packing when spun for at least 10 minutes. An increase in time to 30 minutes will cause a decrease in the highest values of about 1 vol.% A comparison with Wintrobe's method (heparin as anticoagulant) using venous blood, showed a close relationship between the two methods with about 1 vol.% lower values in the micromethod. There also seems to be insignificant difference between simultaneous capillary and venous packed cell determinations, even in a child with a hematocrit of 77 vol.% Neonates were not included in this comparison.

The number of red blood cells were determined using a Zeiss counting chamber ad modum Bürker in the youngest infants, and a Ljungberg Cellоscope at the age of 1½–3 years. There was a close relationship between the figures obtained using the two different methods, with a 1 6% difference in the means. The number of red cells at the age of 1½–3 years has been corrected to correspond to the counting technique

The standard error of a single determina-

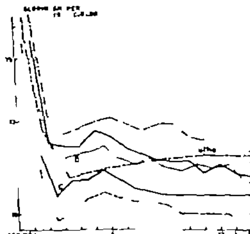


Fig. 1 The author's curve for mean hemoglobin values in g/100 ml compared t the 5 large "normal series from the literature. The curves are from the following authors A Usher and co-workers; B Mackay; C Kato; D Elvehjem and co-workers; E Merritt & Davidson. (Moe [16], page 13).

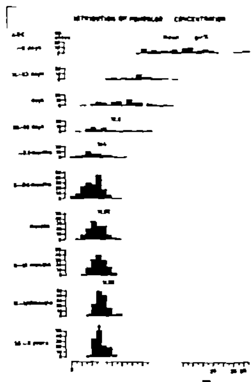


Fig.

tion for the oxyhemoglobin and cyanmethemoglobin methods based on duplicate determinations was 2.6% (2% for the cyan methemoglobin method) 1 6% for the hematocrit, and 3.5 % for the red cells counts using the Zeiss counting chamber The last figure is based on 50 duplicat determinations, and is probably too low as d plicat counts could only be performed on days with few counts.

Methods have been described more completely in the monograph (Moe [16])

Results and Discussion

Hemoglobin

Table 1 shows the mean hemoglobin concentrations during the first three years of life in our studies and in a few investigations from the literature In the figures from the literature small adjustments in the age had to be made in order to compare with our figures.

Our numbe of observations at the different age levels during the first tw months of life ought to have been much higher in order to get exact means, but this is relatively unimportant. It is more important to demonstrate the large range of normal values during early infancy as shown in Fig. . This large range has been shown by other workers, as for instance Elvehjem & Peterson [5] Merritt & Davidson [14], Guest [8] and Vahlquist [22]

Duplicate hemoglobin determinations were not performed in some cases at the different ages during the first three years of life in the beginning of the study. Single oxyhemoglobin [illegible] using the Ljungberg Col[illegible] performed owing to [illegible] stances Those values [illegible] cluded n the present [illegible]

TABLE 1 *Mean hemoglobin concentration during the first three years of life* (g per 100 ml)

Age	Own investigation			Drucker [4]		Vahlquist [22]			Selander [20]		
	No. of cases	Mean	S.D.	No. of cases	Mean	No. of cases	Mean	S.D.	No. of cases	Mean	S.D.
2–6 days	68	19.8	.4			21	19.4	1.64	240	20.4	2.12
14–23 days	20	16.7	1.5	6	17.0	22	17.5	2.10			
24–37 days	59	14.1	1.9	6	14.4						
38–60 days	40	12.8	1.9								
¾ months	45	11.4	1.1	6	10.9	24	12.4	1.14	125	12.0	1.20
3–3½ months	165	11.16	0.83						130	11.65	0.87
4–7 months	129	11.65	0.70	19	11.5				128	10.93	0.49
8–10 months	133	11.76	0.64	16	11.2	23	12.33	1.19			
11–13½ months	134	11.92	0.57	6	11.0				119	11.43	0.90
1½–3 years	121	11.9	0.63			17	12.15	1.11			
Adult males	*7	14.6				50	15.68	0.86			

A closer study of the course of the hemoglobin curve during the first week of life has not been made. Most authors state that there is very little reduction in hemoglobin during the first week of life and this possible reduction is unimportant compared to the large range of normal hemoglobin concentration during this period (Fig 2)

The time of clamping the cord has not been considered, but it is common practice now to delay clamping the cord.

Our number of observations at the different age levels is high from the age of 3 months. From the age of about 8 months, the mean hemoglobin concentration remains about the same and there are narrow ranges of normal hemoglobin concentrations. Mean −2 S.D. will be about 10.5 g/100 ml from the age of 8 months to 3 years. But there is no sharp limit of normal and it is better to use the term minimal zone (Vahlquist [23]) as there is a transitional zone with overlapping of normal and pathological values. All values below this minimal zone may be considered as pathological at the given age while the values in the minimal zone may be normal in some infants and too low in others. In our studies the minimal zone seems to be from 10 to 11 g/100 ml at the age of 8 months and from 10.5 to 11 g/100 ml later on.

There is a close relationship between Selander's and our figure in Table 1 for mean hemoglobin concentration during the first week of life while Vahlquist's mean hemoglobin concentration in venous blood seems to be relatively lower (The difference between Selander's and Vahlquist's mean hemoglobin concentration at the age of 1 day is even as high as 2.5 g%.) The discrepancy between the figures in Table 1 after the first month of life is mainly due to the facts already discussed. Our figures for normal hemoglobin concentrations correspond better with those in recent American publications (Moe [16] page 37) Small differences will probably always exist between figures in different investigations, even using excellent standardization of technique. This

TABLE 2. *Mean hematocrit values (Vol. %) during the first three years of life.*

Age	Own investigation			Drucker [4]		Vahlquist [22]			Mugrage & Andresen [18]			Wintrobe [4]	Mlale [18]
	No. of cases	Mean	S.D.	No. of cases	Mean	No. of cases	Mean	S.D.	No. of cases	Mean	S.D.	Mean	Mean
2–6 days	73	65.9	.5			21	58.2	4.1				53	56–53
14–23 days	31	52.0	5.1	6	54.8	[illegible]	53.7	7.0	22	43.3	9.1		
24–37 days	67	45.3	6.5	6	46.6								
38–50 days	41	42.1	5.9										
2–2½ months	45	37.7	3.6	6	35.7	19	37.0	3.2					
3–3½ months	168	36.7	2.8						22	34.2	2.9	36	
5–7 months	119	35.4	2.3	19	37.9				18	37.4	2.6		36.2
8–10 months	129	36.0	2.2	16	36.1	[illegible]0	35.3	3.8	25	34.8	1.4	35.5	35.5
11–12½ months	129	39.3	1.9	6	34.3							35.0	
1½–3 years	128	38.9	2.3			17	37.9	3.1	22	35.5	[illegible]		
Adult males	27	45.2					47		40	48.4	2.2	47	47

may be due to the personal factor the small inaccuracy in constructing a calibration curve even in a good colorimeter and small changes from time to time in the colorimeters.

Hematocrit

Little ttention has been paid to packed cell volume in most of the studies of the red blood picture in infancy and it is difficult to find comparative figures to ours (Table 2)

Our mean hematocrit during the first days of life is considerably higher than the other figures cited in Table _, but the latter are presumed to be from studies using venous blood. The difference between Vahlquist s and our figure for mean packed cell volume is not more than the cor responding difference in mean hemoglobin concentration. Wintrobe s nd Mlale s figures for mean volume of packed cells during the first week of life are only about 6 vol.% (13 .) bove their mean figures in adult males. This is a small difference considering the fact that the mean hemoglobin concentration during the first week of life in venous blood, is about 3.5 g% (22 %) above that of adult males and the mean MCHC in newborn and older infants is not significantly higher than in adult males [15]. Vahlquist s mean packed cell volume in infants aged 6 days, 58.2 is 11 vol.% above that of adult males.

Hagberg & Lundström s [11] mean he matocrit in 17 infants less than _4 hours old, 62._, is not far below our figure during the first days of life, considering the large range of normal values during that age period. Capillary blood was obtained through pin-pricks in the heel or big toe The mothers of the infants had received intravenous iron. About 90 % of our mothers had received iron therapy

There i a close relationship between the various figures in Table _ for mean hematocrit at the age of 3 months.

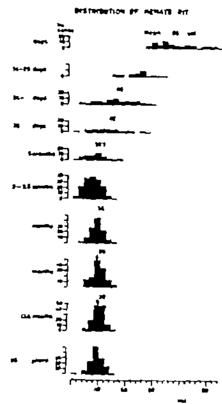


Fig. 3

further drop in most standards may be due to the influence of cases of iron-deficiency anemia during late infancy More recent American studies have shown a similar rise in the mean packed cell volume to that in our studies, with a closer relationship in absolute figures. The close correlation between Sturgeon's [21] and our mean hemoglobin concentration and MCHC must mean that the packed cell volumes are similar

Fig 3 shows the distribution of packed cell volume at different ages. Comparative figures have been found in the studies by Mugrage & Andresen [18] some 30 years ago. Mugrage & Andresen also demonstrated a large range of normal packed cell volume during early infancy, although the absolute values are incomparable. Their mean hematocrit at the age of 1 year is about 12 vol.% below their mean for adult males, while a difference of 6 vol.% was observed in our studies. The mean hematocrit remains about the same in our studies from the age of 9 months with approximately the same range of normal. Mean ±2 S.D will give a range of 34.5 to 43.5 The minimal zone of packed cell volume is wider than the corresponding zone of hemoglobin concentration, and it is difficult to give exact figures. For practical purposes I will suggest a minimal zone of 37–34 from the age of 9 months. Hematocrit determinations are used in *other clinical disorders* than anemia, especially in cyanotic heart diseases and dehydration, and it may be desirable to establish upper limits, or maximal zones, at the different ages. Dehydrated infants are often also anemic. This makes the interpretation of absolute values of packed cell volume difficult. It is more important to use the packed cell volume as a guide for rehydration. No hematocrit values above 44.5 were observed in children more than 3 months old (505 observations), and this may be considered the upper normal limit of packed cell volume

Red blood cells

Table 3 shows the number of red blood cells at the different ages.

There is a pretty good relationship between the various figures for mean RBC in Table 3 except for the high values given by Guest [8] during late infancy (4.8–4.9 mill/mm³) The latter are almost as high as during the first ten days of life. The very high figures reported by

TABLE 3 *Mean number of red blood cells (Mill./mm³) during the first three years of life.*

Age	Own investigation			Faxén [7]			Selander [20]			Mugrage & Andresen [18]			Guest [8]
	No. of cases	Mean	s.d.	No. of cases	Mean	s.d.	No. of cases	Mean	s.d.	No. of cases	Mean	s.d.	Mean
2–6 days	85	5.40	0.65	23	5.34	0.58	249	5.61	0.75				5.–
14–23 days	33	4.93	0.66										
24–37 days	72	4.35	0.57	36	4.70	0.41				22	4.23	0.66	
38–50 days	43	4.10	0.48										
2–2½ months	54	3.75	0.49	66	3.91	0.37	125	4.03	0.53				3.8
3–3½ months	184	3.88	0.42	44	3.96	0.29	139	4.01	0.41	23	3.90	0.40	
5–7 months	129	4.21	0.45	42	4.57	0.35	128	4.16	0.31	27	4.23	0.30	4.4
8–10 months	132	4.35	0.41	25	4.43	0.33				18	4.28	0.22	
11–13½ months	131	4.44	0.41	42	4.58	0.45	119	4.37	0.40				4.8
1½–3 years	125	4.45	0.35							22	4.36	0.21	4.9–4.7

Horan [12] for red blood cells during infancy beginning with 6.54 mill/mm³ are probably beyond any comparison today.

Faxén's, Selander's and our standard deviations are about the same. Mugrage & Andresen's standard deviations during late infancy are amazingly small even considering the fact that they performed their studies on venous blood from a small number of infants. (Their standard deviation at the age of 3 months is the same as ours.) Fig. 4 demonstrates the distribution of the number of red blood cells at the different ages. Guest found almost the same distributions performing his studies on venous blood, while smaller ranges were found by Mugrage & Andresen. The number of red blood cells alone is of relatively little practical importance.

Red cell indices

Wintrobe's red cell indices have been in common use for more than 20 years, and their preference over the arbitrary indices (color and saturation indices) should be obvious.

Tables 4, 5 and 6 show the means of

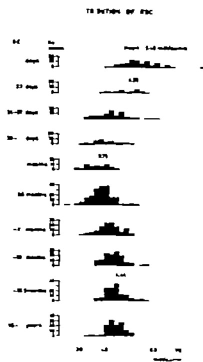


Fig. 4

TABLE 4 *Mean MCHC (%) during the first three years of life.*

Age	Own investigation			Vahlquist [22]			Mugrage & Andresen [18]			Guest [8]		Sturgeon [21]		Whitby [21]
	No. of cases	Mean	s.d.	No. of cases	Mean	s.d.	No. of cases	Mean	s.d.	No. of cases	Mean	No. of cases	Mean	Mean
2–6 days	64	30.0	2.7	21	32.3	1.0				80	34.5			33
14–23 days	27	30.1	_0	22	32.8	1.0								
24–37 days	53	31 1	2.6				23	32.6	0.9					
38–59 days	37	30.3	2.4											
2–2½ months	38	30.1	1 7	19	32.4	1 4				68	34.8			
3–3½ months	147	30.4	1 6				23	32.5	1.3					34
5–7 months	119	30.0	1.5				27	32.8	1 0	103	33.7	96	30.3	
8–10 months	130	30.2	1.3	*0	32.3	2.2	18	33.0	1 1					33
11 13½ months	130	30.3	1.3							153	31.9	93	30 6	32
1½–3 years	128	30.3	1.5	17	32.1	1.5	22	33.0	1 1			91	30.7	32–33

MCHC MCH and MCV and their standard deviations The low hematocrits in older standards (page 60) will result in higher figures for MCHC and lower figures for MCV The high numbers of red cells in some studies [1 11 14, 21 and 8, 22 during late infancy] will decrease the figures for MCH and MCV

Table 4 shows a close relationship between Sturgeon's ("Normal superior group") and our MCHC values.

The trends followed by most of the figures in the different series in Tables 4, 5 and 6 are similar although the absolute figures are incomparable There is no significant difference between the mean MCHC during the first week of life and later on. Actually there seems to be a general consensus of opinion that the mean MCHC remains close to constant during infancy and childhood, except for a possible decrease during late infancy

TABLE 5 *Mean MCH (µg %) during the first three years of life*

Age	Own investigation			Vahlquist [22]			Faxén [7]			Mugrage & Andresen [18]			Guest [8]		Whitby [21]
	No. of cases	Mean	s.d.	No. of cases	Mean	s.d.	No. of cases	Mean	s.d.	No. of cases	Mean	s.d.	No. of cases	Mean	Mean
2–6 days	66	36.7	3.4	21	36.3	2.0	18	42.2	2.7				80	34.7	37
14–23 days	20	31 6	_6	22	35.3	2.4									
24–37 days	58	32.4	2.8				38	38.2	3.3	22	34.0	3.1			
38–60 days	39	31 1	3.0	22	29 8	_9									
2 2½ months	45	30.3	2.8				65	34.6	2.7				68	32.0	
3–3½ months	163	6.8	2.6				43	32.2	2.4	22	28.5	2.1			37
5–7 month	129	27.3	2.8				43	*9.5	2.8	27	29.0	1.7	10*	27 1	
8–10 months	133	27 0	2.5	23	5.4	3.1	25	29.8	2.5	18	27.5	2.1			34
11–13½ months	133	26.9	2.3				43	29 4	2._				153	28.0	33
1½–3 years	1._6	26 6	2.0	17	25.1	2.8				22	28.3	1 4			29

TABLE 6 *Mean MCV* (μ^3) *during the first three years of life*

Age	Own investigation			Vahlquist [22]			Mugrage & Andresen [18]			Guest [8]		Wintrobe [24]
	No. of cases	Mean	S.D	No. of cases	Mean	S.D	No. of cases	Mean	S.D	No. of cases	Mean	Mean
2–8 days	2	122	12.8	21	108.8	8.9				89	107	105
14–23 days	31	106	11.2	22	108._	7.8						
24–37 days	67	104	10.6				2_	103	9.5			
38–60 days	40	103	10.8	19	89.8	8.7						
2–2½ months	48	101	9.6							88	92	
3–3½ months	165	94.5	9.1				22	87.2	5.0			80
5–7 months	119	91.1	9.2				27	88.2	5.7	102	81	
8–10 months	129	89.5	8.1	20	78.6	8.2	16	86.0	3.6			77
11–13½ months	1 6	88.7	7.1							153	71	78
1½–3 years	122	87.3	7.0	17	78.1	6.6	22	88.2	4.2			77–80

caused by cases of iron-deficiency anemia (Guest's figures). There is also general agreement upon the marked drop in mean MCH and MCV during early infancy (Guest's very low figures for MCH and MCV during late infancy must also be due to the influence of cases of iron deficiency anemia). Figs. 5, 6 and 7 show the distribution of MCHC, MCH and MCV at the different ages during the first three years of life. There are smaller variations in MCHC than in MCH and MCV. Similar distribution patterns may be seen in the studies by Guest [8], considering the influence of iron-deficiency anemia during late infancy, and to a lesser degree in the studies by Mugrage & Andresen.

Mean ± 2 S.D. for the three cell indices in our studies will be about 27–33 from the age of 8–10 months for MCHC, 22–32 at the age of 8–10 months and 22.5–30.5 at the age of 1½–3 years for MCH, and 73–105 at the age of 8–10 months and 73–101 at the age of 1½–3 years for the MCV. Only a few of the cases of iron-deficiency anemia in this study [16,

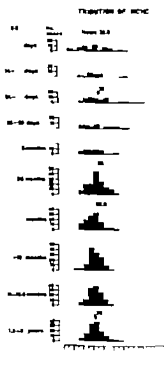


Fig. 5

the age of 8 months, there are narrow ranges of normal hemoglobin concentration and hematocrit. The hemoglobin concentration offers the most sensitive criterion for the existence of iron-deficiency anemia. The large overlapping of normal and pathological values of cell indices should be a warning against the uncritical interpretation of cell indices often seen in practice. One must also bear in mind the large drop in MCH and MCV during early infancy and the subsequent increase during childhood.

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Addendum

Similar distributions for the number of red blood cells at the ages of 1, 2, 3 and 6 months were found in recent study using duplicate determinations in a Ljungberg Celloscope.

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Department of Pediatrics
Haukeland Sykehus
Bergen
Norway

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From Kaptein W Wilhelmsen og Frues Bakteriologiske Institutt Oslo
Head Professor dr med. S. Dick Henriksen

The Frequency of Positive Dye Test in Children from Different Parts of Norway

by TORE MIDTVEDT

Since the discovery by Wolf & Cowen [15] that Toxoplasma gondii is a cause of human disease numerous reports have appeared describing the frequency of toxoplasmosis in man from different parts of the world. It has been shown by many authors that the parasite has a world wide geographical distribution and infects many species of birds and mammals including man.

The frequency of positive dye test (DT) in man is found to vary in different climate zones—it seems to be higher in the temperate and tropical zones than in the arctic one As a rule the frequency of positive DT is higher in rural areas than in urban ones. That suggests possible connection with farm animals.

But as Beattie [1] pointed out in spite of the large amount of work that has been done especially in the last ten years, much remains to be done about the epidemiology of toxoplasmosis

It has been the purpose of the present investigation to study the frequency of positive DT among children from different geographical part of Norway

Materials and Methods

Sera were taken from children in the following three groups:

I The first group consisted of 100 school children from the Lapp minority in Finnmark, the most northern county in Norway

II. The second group consisted of 115 schoolchildren from Finnås, a small island in the south-western parts of Norway

III. The third group were children from different departments of Rikshospitalet, Oslo They were from the whole country except Finnmark. Children with a history or present illness suggesting toxoplasmosis were not included.

The DT technique was in the main as previously described [16] except that the reactions were considered as positive when more than 50% of the extracellular toxoplasmas in titer 1 4 or higher were unstained.

Results

The sera from the schoolchildren, 6–14 years old, were divided into two groups, 6–10 and 11–14 years old. As shown in Table 1 there were about the same percentage of positive DT in both the age groups in the sera from Finnås a in those from Rikshospitalet In contrast to these

TABLE 1 *The frequency of positive dye test in children from different geographical parts of Norway*

Group	Age in years	No. of sera	No. of positive	No. of negative	Per cent of pos.	Age group in per cent	Per cent of pos. in whole group	
I	6–10	32	0	32	0	32	5	
	11–14	68	5	63	7	68		
II	6–10	43	6	37	14	37	16	15.5
	11–14	72	12	60	17	63		
III	6–10	40	5	35	13	47	15	
	11–14	45	8	37	17	53		

I. Children of Lapp origin from Finnmark.
II: Children from the island of Finnøis.
III: Children from Rikshospitalet.

the frequency of positive DT in sera from both age groups of Lapp children were remarkably lower As a whole the frequency of positive DT in the Lapp sera was 5% compared with 15.5% in the other sera. In these two groups the observed and expected frequencies were of sufficient size to permit a comparison by the X^2 test. Calculations gave the value $0.02 > P > 0.01$ Thus the observed difference seemed to be statistically significant

Table 2 presents the titers of the positive DT in the three groups of children. The values seemed to be somewhat higher in the two first groups than in that from Rikshospitalet but the differences were too small for evaluation.

Discussion

As mentioned, the frequency of positive DT in the Lapp children is found to be significantly lower than in children from other parts of Norway Many factors, such as climate nutrition, communications, medical service etc. may be different in Finmark. The Lapps are the aborigines of the subarctic counties in Scandinavia. Some of them are now living as the other inhabitants, but many are still nomads in the mountains. In a few summer months the latter live with their reindeer on islands along the coast in the rest of the year they live in the inland. Here it is a typical subarctic inland climate Average air temperature during a year is +2 1 C and the

TABLE 2 *Titre values in the positive dye tests*

Group	Titre 1:4	1:10	1:40	1:200	1:400	>1:400	Total
I	—	2	1	1	1	—	5
II	1	5	7	2	2	1	18
III	5	3	4	1	—	—	13
Total	6	10	12	4	3	1	36

The groups are the same as in Table 1.

TABLE 3 *Serological investigations of toxoplasmosis in countries in the temperate climate zone*

Author(s)	Country	Material	Method	Age (years)	N of tested	Positive in %
Keller & Vivell [9]	Germany	Normal	DT	1 10	82	17
		Pos. ≥ 1/25		11–20	127	[illegible]0
Thalhammer [13]	Austria	Normal	DT	6–10	66	9.0
				11 [illegible]0	6	36.8
Thalhammer [14]	Austria	Normal	DT	6–10	125	7.4
				11–14	43	13.9
French & Fish [4]	Canada	Normal	DT	3–9	45	4.4
				10–19	6[illegible]	17.2
Hagberg [8]	Sweden	Normal	Skin test	–8	114	8.8
				9–10	174	14.9
				11–1[illegible]	99	16.[illegible]
				13–14	43	18.6
Gard [5]	Sweden	Normal	Skin test	8–10	156	14.1
				11 15	128	16.4

precipitation is 316 mm [1] About 75 % of the children in the present material came from nomadic families, the others came from small farms in the inland.

The low frequency of positive DT in the Lapp group is surprising in view of the close contact of these children with domestic (reindeer dogs) and wild animals, and their often poor nutritional and social conditions, both of which factors might have been expected to cause a higher frequency of toxoplasma infection. Of the factors which might be thought to explain the low frequency of toxoplasma antibodies in the Lapp children, the climate is the most striking one There are however very few reports on the frequencies of these antibodies in the northernmost parts of Scandinavia

In an investigation with t xoplasmin skin test (ST) on adults living in Boden a little town in the northern part of Sweden, 9 8 % of the tests were found to be positive as compared to about 45 % in the southern parts of Sweden [5 11]. In 24 cases of uveitis from the Eye Department Troms and Tromsö hospital in the northern part of Norway Bergaust found only one case with a positive DT [ ]. It has to be mentioned that the DT was not carried out frequently in the latter investigation. Bergaust pointed out that no case of toxoplasmosis has as yet been published from the northern counties of Norway In contrast to the low frequency of positive DT in the material recorded by Bergaust an investigation of 58 cases of horioretinitis from Rikshospitalet, Oslo, showed a frequency of 67 % of positive DT The frequency of positive DT in th control group was found to be 28 % [6].

Reports from other countries with subarctic climate are also very scanty In an investigation among 21 Alaskan Eskimos Feldman & Miller [3] found no sera with positive dye test In Iceland where the climate is nearly subarctic the same au thors found 11 positive DT in 108 sera from adults.

As far as is known, the investigations

mentioned above are the only ones dealing with the occurrence of antibodies against Toxoplasma gondii in people living in subarctic regions yet published. All of these investigations seemed to be in agreement with our findings thus showing a lower frequency than in the temperate climate zone

The climate in Finnås is typical coastal with mild winters and wet summers Most of the people live as fishermen and farmers The children in Rikshospitalet are from all over the country and with parents of different occupations. Except for the most northern part Norway belongs to the temperate climate zone

Epidemiological studies of toxoplasmosis in people living in the temperate climate zone are far more numerous than from the arctic zone There are also many such investigations in children and some of the results are reviewed in Table 3

In addition a preliminary report of a study in the incidence of toxoplasma antibodies in 2305 people living in Sundbyberg near Stockholm also has to be mentioned [9] Antibodies were found in 3–4 % in children aged 1–5 years raising to about 19 % among the oldest school children

Such a comparison of DT and even toxoplasmin cutan tests by different researchers has to be taken with many reservations as mentioned by Grönroos [7]. However there seems to be a good correspondence between our findings of positive DT in children from the southern parts of Norway and other investigations from the *temperate zone*

In the material from Finnås, 4 of the 5 sera with DT titer above 1 40 were from children living in the neighbourhood of each other No further investigations were carried out, but it might be taken as an indication of recent infection from the same source

Summary

The frequency of positive dye test in children living in different geographical parts of Norway has been studied. The frequency of positive tests in children living in the subarctic climate zone was found to be 5 % compared with 15.5 % in children living in the temperate climate zone The difference was found to be statistically significant at the 2 % level.

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Wenner-Gren Center
Sveavägen 166
Stockholm
Sweden

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CASE REPORT

Neonatal Leucopenia due to Fetomaternal Leucocyte Incompatibility

by KARIN HALVORSEN

From the Pediatric Department, Oslo City Hospital (Head Rould Bloch, M.D.)

The formation of leucocyte agglutinins by iso-immunization has been reported by several authors. Most reports deal with the leucocyte antibodies produced following multiple transfusions. Less known is the leucopenia in the newborn infant caused by transplacental transfer of maternal leuco-agglutinins.

The present paper reports two cases of neonatal leucopenia due to leuco-agglutinins. The iso-immunization in the mothers has probably been due to previous pregnancies and blood transfusion.

Method

The presence of complete leucocyte agglutinins was investigated according to the technique described by Dausset [3] using a dextran solution (Intradex "Nyco") instead of a polyvinyl-pyrrolidone solution. Freshly drawn blood was defibrinated and mixed with one-fifth its volume of Intradex. The mixture was left at 37 C in an inclined tube for 30 min and the pink supernatant pipetted off—*containing 2000–5000 WBC/mm*

One-tenth ml of the mother's serum previously inactivated at 56 C for 30 min was mixed with 0.05 ml of white cell suspension. Further tests were set up with a 1 in _ and 1 in 4 dilution of the mother serum in saline.

The mixtures were left 90 min at 37°C, then 0.1 ml of 1% acetic acid was added to hemolyse the red cells, and a thick drop of the mixture examined microscopically.

Case Reports

Case 1

E. R., a 1-hour-old boy was admitted to the Children's Department Oslo City Hospital, February 11th 1963, because of erythroblastosis. He was the second child of a healthy mother who had never received blood transfusion. The mother's blood was group A, Rh negative. In the first pregnancy Rh antibodies were not present and the child is healthy. In this pregnancy Rh antibodies, anti D were demonstrated 1 month before term with titers 1024 in albumen and 16.4 by indirect Coombs technique. Because of the high titers Cesarean section was performed 3½ weeks before term.

On admittance to the Children's Department the infant was weak, pale and edematous. The cord was yellow. The liver and the spleen were palpable 1 cm below the costal margin.

Laboratory investigations. The hemoglobin was 12.9 g/100 ml, the reticulocytes 2.0%, the leucocytes 9300 and the differential count showed 4% eosinophils, 61% segmented granulocytes and 35% lymphocytes. The serum bilirubin was 6.2 mg/100 ml. The direct Coombs test was strongly positive

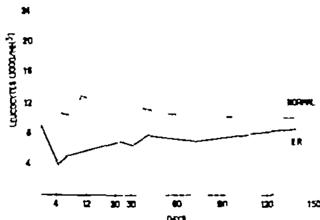


Fig 1 Number of leucocytes in peripheral blood in Case 1 (E. R.) compared with the mean of normal values.

A diagnosis of severe erythroblastosis with possible hydrops was made, and an exchange transfusion was performed immediately and repeated 6 hours later The leucocytes were followed every other day and were on the fifth day 3800 and the seventh day 4900 The differential counts were unchanged Fig 1 shows the number of WBC during the following weeks, indicating normalization of the values from 4 weeks of age. The mother had normal WBC and LE cells were not demonstrated.

Serum from the mother was examined for leucocyte-agglutinins against leucocytes from the f ther Later when the patient was 6 weeks ld, the mother serum was examined against the patient's leucocytes. The results are shown in Table 1. Leucocyte-agglutinins were demonstrated in the serum from the mother which agglutinated leucocytes from the father but however not her own leucocytes A weakly positive reaction was found between serum from the mother and the leucocytes from the patient.

TABLE 1 *The results of the leucocyte agglu t nation tests performed with serum from Case 1 (E R) and his mother*

		Dilution of serum		
Serum	WBC	1/1	1/2	1/4
Mother	Mother	–	–	–
Mother	Father	+ +	+	+ (+)
Mother	Infant	–	(+)	–
Infant	Infant	–	(+)	–

Case 2

C R. 2-day-old boy was admitted to the Children Department March 14th 1962 because of weakness and congenital anomal es. H was the fourth child f a healthy group A, Rh positive mother The three previous children were healthy Following the last hildbirth in 1956, the mother had an abortion and received a blood transfusion. There was no immediat reaction to the transfusion.—The membranes ruptured 10 minutes before the delivery and the amniotic fluid was discolored. The delivery was normal, the birth weight 3160 g and the length 51 cm.

On admittance to the hospital he was weak and cried faintly There was a marked micrognathia. The body was long and slender the arms appeared unusually long The knee joints lacked "0" on full extension and he had a moderate pes equino varus on the right foot. The testicles were not

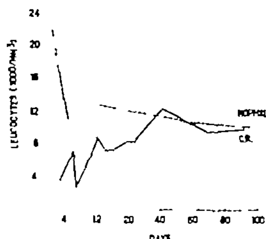


Fig. — Number of leucocytes in peripheral blood in Case (C. R.) compared with the mean of normal values.

descended. There was a generally increased growth of hair

Laboratory investigations The hemoglobin was 24 6 g/100 ml and the reticulocytes 1.3%, the thrombocytes 239 000 the leucocytes 2600 with 1% myelocytes, 8% eosinophils, 72% segmented granulocytes, monocytes and 14% lymphocytes. The number of nucleated red cells were 3/100 WBC. The serum proteins were normal. The urinary amino acid excretion and 17 ketosteroid excretion were normal. The cerebrospinal fluid was normal and the bone marrow showed active myelopoiesis and was other wise normal. He had a normal masculine chromosome picture

The number of leucocytes was followed closely and was 7000 the third day 2800 the sixth and 4800 the eighth day with un changed differential counts. Fig 2 shows the further course of the leucocyte values, indi cating a steady rise towards normal values.

The mother had 8200 WBC and LE cells could not be demonstrated in her blood. Serum from the mother was examined on the presence of agglutinins against the leucocytes from the patient and the father Table 2 shows the results of these tests.

The results indicate that the serum from the mother contains leucocyte antibodies which agglutinated leucocytes from the patient and the father

Discussion

The formation of leuco-agglutinins following multiple transfusions has been demonstrated several times. More recently it has been shown that leucocyte antibodies can be produced by immunization during pregnancy van Rood *et al.* [12], Payne & Rolfs [10] and Jensen [6] demonstrated that leucocyte antibodies can be formed during pregnancy The number of immunized women seems to increase with the number of pregnancies In non transfused primiparae no leuco-agglutinins could be demonstrated. Jensen [6] demonstrated complete leucocyte antibodies in the cord blood only when the maternal titre was higher than 1 16 In a recent study Payne *et al* [11] examined the number of leucocytes in newborn infants of mothers who had leucocyte-agglutinins in their serum. The authors did not find support for the existence of an immuno-leucopenia of the neonatal period. Their data show how

TABLE 2 *The results of the leucocyte agglutination tests performed with serum from Case 2 (C R) and his mother and father*

Serum	WBC	Dilution of serum 1/1	1/2	1/4
Mother	Mother	–	–	–
Mother	Father	+ + +	+ +	+
Mother	Infant	+ (+)	+ (+)	–
Father	Father	–	–	
Father	Mother	–	–	–
Infant	Infant	–	–	–
Infant	Father	–	–	–
Infant	M ther	–	–	–

ever lower leucocyte counts shortly after birth in the experimental group than in the control group. Low leucocyte-agglutinin titer may explain the lack of convincing leucopenia in their study.

Leucocyte antibodies are most likely produced in the mother just as the erythrocyte antibodies in hemolytic disease of the fetus and newborn. Leucocytes pass from the mother to the fetus [4], and they probably also pass in the opposite direction.

Neonatal leucopenia due to maternal iso-immunization has been demonstrated a few times. The condition was first suggested by Luhby & Slobody [9], however without demonstration of the antibodies in the actual cases. Hitzig & Gitzelmann [5] found complete leucocyte-agglutinins in a 6-week-old child with leucopenia and low resistance to infections. Leucocyte antibodies showing identical agglutination reactions were found in the serum of the mother, however with higher titer.

Lalezari *et al.* [8] reported three siblings with marked leucopenia, granulocytopenia and severe infections in the neonatal period. Leucocyte agglutinins could be demonstrated in the serum of the infant and the mother—in the infant up to the age of three weeks. Jensen [6] reported a newborn infant with severe malformations, leucopenia and infection. The infant was the fourth child of a healthy mother. In the serum of the mother and the infant identical leucocyte-agglutinins could be demonstrated. Braun *et al.* [2] reported three siblings with neonatal neutropenia and infections. Leucocyt antibodies were demonstrated in the serum from the mother and in cord blood. The mother had previously received a transfusion with her husband's blood.

In some of the reported cases with neonatal leucopenia due to maternal iso-immunization, the differential count has been normal, in others a granulocytopenia has been present. Some cases have had severe infections ending fatally, others have had no symptoms. Leucocyte-agglutinins have not been demonstrated in all infants. Leucocyte-agglutinins have not been found in the author's two infants. The reason may be that the antibodies are attached to the leucocytes of the child after passing placenta.

The most important differential diagnosis in the leucopenic newborn infant is leucopenia due to severe infection. The author has observed a patient with coli sepsis in whom the leucocytes were 2000 at the age of three days. No leucocyte-agglutinins could be demonstrated in the serum from the mother against leucocytes from the father. Virus infections may also cause leucopenia. Kostman has reported a congenital, hereditary agranulocytosis. Lupus erythematosus in the mother [13] and other causes of auto-immune leucopenia in the mother [14] may cause leucopenia in the fetus due to passive transplacental transfer of antibodies. In the Swiss type of a-gammaglobulinemia there is marked lymphocytopenia, no increase in leucocytes with age and the infants die early from infections.

In our Case 1 the iso-immunization is probably due to a previous pregnancy. The patient had Rh-erythroblastosis. Ballowitz & Ballowitz [1] reported a case with erythroblastosis and leucopenia. There is some evidence for the existence of AB0 and Rh factor in the leucocytes and it is possible that the leucocytes share common antigenes with the erythrocytes [15]. It is an interesting observation that one of the present cases also had Rh-erythroblastosis, and the presence of leucopenia

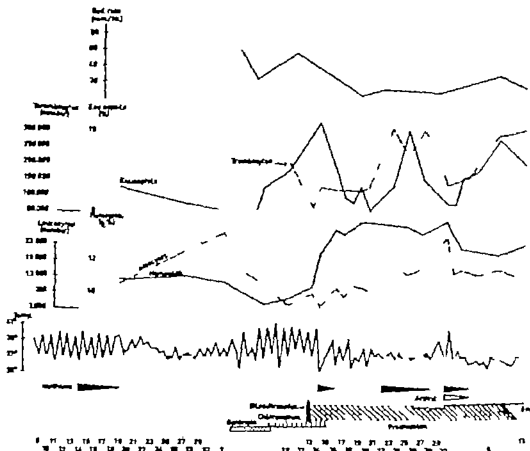


Fig 1

On Oct. 14 it was observed that the lymph nodes of the neck had increased to bean size with some oedema around. A pea-sized adenitis in the left armpit and groin. Lymph node biopsy was performed but showed only general irritation, nothing specific A spotty exanthema reappeared on the trunk but disappeared in a couple of days.

The condition improved, the temperature fell and the patient ate better On Oct 22 an exanthema was again observed spread symmetrically over the entire body in the form of pinhead-and confetti-sized dark red maculopapules, with butterfly-shaped erythema over the nose and cheeks.

The patient's condition improved, the exanthema faded and she remained afebrile for a week. But then a new febrile period started, with exanthema of garland type over thighs and buttocks, around elbows and navel. At the same time the right knee-joint became swollen and its mobility was restricted. On this occasion, however her temperature fell quickly and the exanthema and arthralgic symptoms disappeared within a few days.—Prednisolone therapy which had been proceeding since Oct. 13 was gradually diminished and was stopped on Dec 5.

In the latter half of December the patient had a fourth fever period with maximum temperature 38.1 lasting one week, but otherwise no symptoms. Under continued observation during 196_ and 1963 she has been healthy

Laboratory Findings

(See Fig 1)

Peripheral blood picture. Anaemia developed during the second fever period. The leucocytes, which had numbered 10,500 on admission, rose at the start of the second fever period on Oct. 2 to 26,100. The differential picture then showed 3% staffs, 68% segmented, 26% lymphocytes, 3% monocytes. During the subsequent course of the fever the leucocyt count fell as low as 3600 on Oct. 14 with 21% staffs, 30% segmented 11% eosinophils, 36% lymphocytes, 2% monocytes. At the same time the number of thrombocytes fell from 209 000 to 68,000. In the third fever period she again had leukocytosis up to .5,300 The thrombocytes were again down to 1?9 000. After the third exanthematous stage she had again a 10% eosinophilia.

The *sed mentation rate* remained very high, bout 65 mm/1 hr by micro method (equal to bout 100 mm macrometrically), until after the second febrile period, when it rapidly fell during the treatment with corti sone

Electrophoresis showed initially a low albu min content, high α_2-globulin and slightly elevated β-globulin, but normal γ-globulin.

	Albumin	Alpha$_1$	Alpha$_2$	Beta	Gamma	Total protein
Sept. 26	3.07	0.33	1.31	0.98	1.11	6.60
Oct. 11	2.90	0.25	0.73	0.82	1.10	5.90
Dec. 28	4 01	0.20	0.50	1.04	1.19	7.0

Acute phase protein. C reactive protein (CRP) was strongly positive on Oct. 10 and 31

Bacteriological tests. Blood cultures on Sept. 22, Sept. 27 and Oct. 30 negative

Nasopharyngeal swabs showed ordinary flora on several occasions; haemolytic streptococci were not found in samples collected on sixteen occasions.

Urine cultures showed no growth in five samples.

Virus culture from faeces on Sept. 19 nega tive.

Serological tests

	AS	ASta	APn
Oct. 5	25	0.25	280
Oct. 31	36	0.25	1600

Paul Bunnell, cold agglutination, Widal tests negative as also tests for toxoplasmosis and Listeria.

Other blood tests LE cells not found in five samples. GOT and GPT showed normal values three times.

Urine Albuminuria (0.5 per mille) on two occasions, otherwise nothing abnormal.

Discussion

The patient had a prolonged illness with four febrile periods, the first two with temperatures of strongly remittent type four recurrences of exanthema in varying forms and during one period, arthritis in a knee-joint Leucocytosis with shift to the left was found on several occasions and likewise eosinophilia The sedimenta tion rate was high and anaemia developed. The general condition was in the circum stances only moderately affected and the course finally favourable Thus all criteria for Wissler s syndrome were present

Apart from these symptoms tonsillitis appeared in the early stage and later a mild swelling of the lymph nodes No pathological symptoms were found on any occasion from internal organs (only febrile albuminuria on two occasions)

Bacteriological tests of blood, sputum urine and cerebrospinal fluid yielded negative results, and a thorough serological

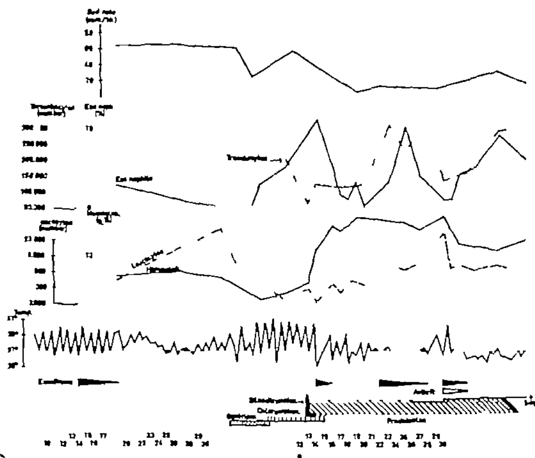


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The *sedimentation rate* remained very high, about 45 mm/l hr by micro method (equal to about 100 mm macrometrically), until after the second febrile period, when it rapidly fell during the treatment with cortisone

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Serological tests

	AS	ASta	APn
Oct. 5	25	0.25	200
Oct 31	34	0.25	1600

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analysis showed as only positive finding a strongly rising titre for antipneumolysin. Virus culture from faeces was also negative

Positive findings in other respects were a high content of acute phase protein (CRP) and lowered albumin, strong rise of $alpha_2$-globulin and moderate rise of beta globulin in the blood

Wissler [16 17] considered the syndrome to be closely associated with but not identical to rheumatic fever or rheumatoid arthritis He assumed that there was a very mild form of sepsis—the bacteria being so few in number as to be undetectable yet causing an allergic-hyperergic reaction. He proposed the name *subsepsis hyperergica*. Fanconi [5] was of the same view but wished to change the name to *subsepsis allergica* Others are opposed to the sepsis theory e.g Denys [4] who instead proposed the name *pseudosepsis allergica*.

It is natural to assume a relationship with the collagenoses. The acute condition may closely resemble rheumatic fever ome cases have developed into Still s lisease or rheumatoid arthritis [4 11 12 16]. Our patient admittedly had no poly arthritis, but did have monarthritis, and on one occasion an eruption similar to erythema annulare high sedimentation rate and strongly positive CRP

Like all other cases described, our patient exhibited a sepsis-like picture—prolonged, high and intermittent fever leucocytosis with shift to the left, high sedimen tation rate The high CRP may also be explained by sepsis [8] as also the changes in plasma protein [1] Polymorphous exan thema and affection of the joints may also be part of the picture Yet in this case as well blood cultures were entirely negative Likewise, the relatively satisfactory general condition, spoke against sepsis. Antibiotics, also were ineffective

The pathological picture can therefore not be included in the rheumatic groups, and evidence of septic affection is lacking. But even if Wissler considered the cause to be a mild septic affection, he believed that an allergic-hyperergic component was an important factor for the clinic and referred especially to the skin symptoms and eosinophilia The general symptoms should also be entirely explainable on the basis of an allergic reaction. Another sign of such a mechanism was the fairly pronounced thrombocytopenia in our patient during two of her febrile periods, a not unusual reaction in allergic states. The leukopenia which followed on the leucocytosis during a certain period may be explained in the same way The eosinophilia was also quite pronounced on several occasions

An allergic mechanism should be of decisive significance The tendency to recurrence is also very marked, as in Vester mark's case [15] of a boy who had no less than ten periods of prolonged illness between the ages of 3½ and 11 years; also in a case reported by Gralain *et al.* [7] in which 36 attacks occurred over a period of nearly 4 years

What is then the cause of the allergic reaction? In several cases it appears to have been a mild infection in the pharynx or upper airways [2 4, 6 17]. In a couple of cases there was a possible connection with a focus of infection in the teeth [5 17] Pathogenic bacteria have generally not been found. Streptococci in the root of a tooth were established on one occasion [5] Böttiger & Landegren found staphylococcus aureus on repeated occasions in the

sputum of one of their two cases, and both had a rising antistaphylolysin titre A high antistaphylolysin titre was also noted at certain periods in Vestermark's case The antistreptolysin titre has been high on a few occasions [6 15] but has usually been normal [2, 4, 6 10 15, the present author] In our case, on the other hand, the antipneumolysin titre showed a sharp rise

In this connection there is reason to devote attention to symptoms in adults which it seems to me are probably of the same category as Wissler's syndrome.

This is perhaps not merely a paediatric problem, as emphasized also by Böttiger & Landegren. Their two patients were 16 years of age Turning now to the literature one finds under the name *Febris maculosa intermittens* [3] the same picture in seven adult patients—prolonged intermittent fever transient exanthema, arthralgic symptoms, leucocytosis. Sepsis was suspected, but blood cultures yielded negative results. These uthors came to very similar conclusions concerning the pathogenesis—a bacteriaemic reaction or an allergic reaction to dissolved substances from some bacterial focus. It is of interest that one case, after an illness of one month, suddenly developed ant meningococcic meningitis. In another case a gonococcic infection was evident and in two others there was justifiable suspicion of gonococcic aetiology Urbach [14] also reports the same symptoms in a gonorrheal patient. Uhse [13] holds it probable that a meningococcic infection was the cause in her case and refers t the same time t French observations of a similar condition which was given the name *Fièvre intermittente pseudopaludéenne*

If one may hazard a conclusion from these cases, it would seem that Wissler's syndrome occurs at all ages. Its nature of allergic syndrome is quite convincing Most observations also appear to me to support the view that it is caused by mild inflammatory processes on different mucosa, especially in the pharynx and upper airways. Aetiologically on the other hand, insofar as the rather few and inadequate investigations permit any conclusion, it seems as though all common bacteria of a coccic nature could be held responsible In the ultimate resort, therefore the mechanism is quite similar to that in rheumatic affections in which some factor from haemolytic streptococci plays a fairly decisive part This, on the other hand, seems seldom to be the case in Wissler's syndrome

Summary

A case of Wissler's syndrome in a 19-month-old girl is described. Clinically there was initially an acute pharyngeal affection and later a mild cervical lymphadenitis. The patient had four febrile periods and as many maculous and erythematous eruptions of different kinds, on one occasion also arthritis in a knee, but no affection of internal organs. Bacteriological and serological analysis revealed as only pathological finding a sharply rising antipneumolysin titre No cytopathogenic viruses were found in faeces. The syndrome must undoubtedly be of an allergic nature as corroborated in the present case by a pronounced eosinophilia, a temporary thrombocytopenia and by a leukopenia superseding leucocytosis. The analogy with febris maculosa intermittens in adults is emphasized. The syndrome quite often seems to be a result of mild inflammatory mucous processes in which various cocci may perhaps play the chief role

a month, referring to the possibility of cerebral hemorrhage [9]. A spontaneous remission is, however, to be expected in most cases.

Epsilon-aminocaproicacid (=Eaca) is reported to be a useful antifibrinolytic and hemostatic drug even in cases where local fibrinolytic activators are assumed to be the cause of the bleedings as in some conditions of hematuria [7, 11]. It has been used in thrombocytopenic diseases but without effect on the thrombocytopenia per se. In these two cases Eaca was used primarily attempting to stop the hematuria. In Case 1 besides fresh blood transfusion and corticosteroids Eaca was given when the hematuria started, and the urinary sediment was normalized within 8 days. In Case 2 the Eaca treatment was removed after 25 days, as the platelet count remained unchanged, but 10 days later the patient had a severe macroscopic hematuria. Within 4 days after restoring the Eaca treatment the urine was normalized. The cause of this hematuria might be the reduced corticosteroid dosage or maybe the discontinuing of the Eaca treatment before the platelet count had been normalized. It is not known whether a hyperfibrinolytic state is present in hematuria in connection with thrombocytopenia, but Eaca might be a useful drug even in this type of hematuria.

Summary

Report of two cases of thrombocytopenia after rubella complicated by purpura, severe epistaxis and profuse hematuria, treated with Epsilon-aminocaproic acid.

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Department of Pediatrics
Barck Children Hospital
Helsingborg
Sweden

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(From the Paediatric Clinic, University of Gothenburg Barnsjukhuset Gothenburg, Sweden)

Cow s Milk Induced Malabsorption as a Precursor of Gluten Intolerance

by S. P FÄLLSTRÖM J WINBERG and H. J ANDERSEN

Cow s milk has long been invoked as an aetiologic factor in certain infants and children with chronic gastrointestinal symptoms [1 5 7 12 23 26 27 30]. In some of these instances the patients have been judged as having coeliac disease However in many of these reports the evaluation of this diagnosis is difficult Firstly the demonstration of malabsorption inter alia of fat and carbohydrates, which is fundamental in diagnosis of coeliac disease is lacking in most instances Secondly in many cases the diagnosis of cow s milk intolerance seems to be based more on the presence of antibodies to cow s milk in the serum of the patients than on n unequivocal demonstration of a deleterious effect f cow s milk. Thirdly the lack of a detailed account of the diet of the patients makes it impossible to exclude the existence in some of these cases of the only recently described hereditary enzymatic deficiencies with intolerance to different mono- and disaccharides [8 10 14 *0 1 _5 34]

To the best of our knowledge only two well documented cases of cow s milk provoked steatorrhoea in infants and children have been published, namely that of DAVIDSON 1958 [9] and that of LAMY FREZAL & REY 1963 [*4]. In the first case there was an intolerance to beta lactoglobulin in the second the factor responsible was not identified but it was not lactose In a patient not yet published, GOLDMAN has shown that casein from cow's milk was capable of inducing steatorrhoea [16]. In dults isolated cases of non tropical sprue with jejunal villous atrophy responding to milk free diet but not the omission of gluten have been reported [31 33].

During the last years we have had the possibility to study four patients in whom a substance in cow s milk—which is not lactose—seems to have been responsible for the development of a malabsorption syndrome It is of special interest that these patients later developed a temporary sensitivity to gluten.

The few earlier descriptions of well documented cases of cow s milk induced steatorrhoea and the association of n early cow s milk intolerance with a later ppearing gl ten intolerance was the reason for this report.

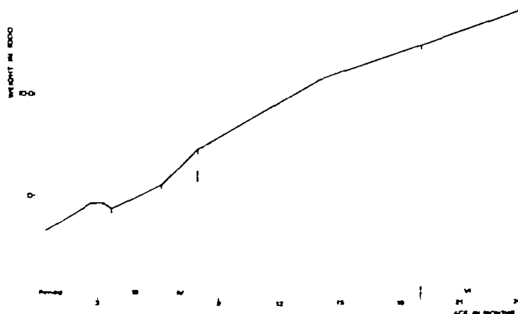


Fig. 1a. Case 1. Weight increase in relation to feeding. Period I: Cow milk, sucrose, wheat or oats. II: Acidified skimmed milk, glucose. III: Breast milk. Several short exposures to cow milk and/or gluten. IV: Breast milk, bananas. V: Cow's milk, rice. VI: Ordinary food, containing gluten.

FAT EXCRETION g/day



WEIGHT CHANGE g/day



STOOLS g/day



VOMITING



EXPOSURE



MAIN FEEDING



AGE IN DAYS



Fig. 1b. Case 1. Weight change, stool weight, fat excretion and gastrointestinal symptoms in relation to main feeding and exposures during hospital stay. Figures under gluten exposure mean g/day.



Fig 1c. Case 1 Age 4 months.

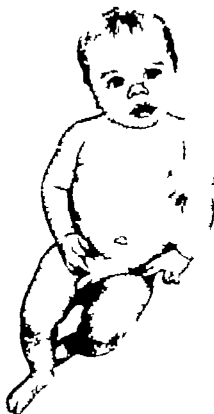


Fig. 1d. Case 1. Age 8 months.

M thods

Xylose in serum and urine was determined according to ROE & RICE [28]. Normal range for serum concentration after an oral load with 2 g of xylose per kg body weight is given by EEG-OLOFSSON [11]. F t content in faeces and milk was estimated according to modification of the method described by KAMER & HUISINGE [22] Otherwise common clinical methods were used.

Case Histories

Case 1 S.Å.L ♂ 600518

Summary The patient was fed cow milk with sucrose and wheat flour from the first month of lif Onset of severe gastrointestinal symptoms t the ge of 3 months. A clinical picture of a malabsorption syndrome with steatorrhoea developed. Disaccharides could be excluded as a cause of the symptoms. Gluten even in large doses caused no immediat symptoms when administered at the age of 5 months. Cow milk repeatedly provoked acut gastrointestinal symptoms. Tolerance to cow milk appeared after the age of 5 months. Intolerance to gluten suc essively developed. During the age of 7–11 months even a single small dose of gluten provoked severe gastrointestinal symptoms. At the age of 1½ years, however tolerance to gluten again ppeared.

Detailed history Fourth child of unrelated parents. Siblings healthy Birth weight

3300 g Breastfed fu t week of life. H was then given a cow s milk formula containing sucrose and from the age of one month wheat flour or oat (Fig 1a, period I) Up to the age of 11 weeks no gastrointestinal symptoms but somewhat slow weight in crease He then started ha ing frequent, loose stools not responding to dietary restrictions and was admitted to hospital at the age of 15 weeks. He was lean and pale with distended abdomen (Fig 1c) There was no dehydration Weight 4450 g. No rickets or anaemia Prothrombin index 66. No pathogens in stools. Chloride concentration in sweat trypsin concentration in faeces and pulmonary roentgenogram normal Electrophoresis normal. Intracutaneous tests with whole cow s milk and with beta and alfa-casein, beta-lactoglobulin and alfa-lactalbu min were all strongly positi e. Precipitating anti bodies to cow s milk were demonstrated in serum by gel diffusion at several occasions (Dr L. Å. Hanson)

H was first treated with acidified skim med cow s milk containing glucose but no cereals (Fig. 1a period II and Fig 1b day 10? 114) No improvement occurred, how ever until cow s milk was replaced by breast milk. His stools now improved successively (Fig. 1a period III and Fig. 1b day 115–122) F t excretion just after transfer to breast milk was 11 g/day during a 3-day period corresponding to an absorption coeffi cient of 68 After 25 days on breast milk fat excretion was 4 7 g/day during a 3-day period and fat absorption coefficient 88 A second exposure to cow s milk without added sugars or cereals in amounts increasing from 100 to 300 g (day 119–122) was ac companied by fever vomiting, voluminous stools and weight reduction. A third exposure to pure cow s milk in amount increasing from 13 g to 80 g per day was performed during the days 141 147 The stools initially became looser and more voluminous but symptoms were not serious as earlier At the end of the exposure the stools became normal and at fourth exposure 2 weeks later he tolerated 1000 g of cow milk per day without an symptoms t all Precipitat ing antibodies to cow s milk were still present in serum t this time

On a diet of breast milk he was at the age of 169 days for the first time exposed to pure gluten and tolerated a total amount of 110 g administered during four days without def nite symptoms. Thus the small amount of gluten contained in his food earlier probably were not responsible for the severe symptoms at the onset of the disease. When gluten was given in combination with cow milk (day 1 6–180) vomitings and more voluminous stools appeared. A third exposure to gluten (day 185)—now together with breast milk feeding—caused an immediate reaction with vomitings, increasing amounts of stools and weight loss. During the whole period of gluten exposures there was a mar ked weight lability (Fig 1b) Fat excretion estimated daily before and during gluten exposure generally amounted to between 5 and 10 g per day (Fig 1b).

At the age of 6 months he was discharged with a diet of breast milk and bananas (Fig 1a period IV) Readmitted at the age of 7½ months for further investigations. He had been well and gained 1670 g (Fig 1a, period IV) The stools had been normal. Fed breast milk he was again exposed to gluten 5 g in one dose (Fig 1b, day 225). After one hour he started vomiting and developed a severe shock. Recovered after epinephrine The following few days he had voluminous stools, maximally amounting to 403 g per day

From this time he was fed a cow s milk formula with rice and sucrose various vegetables, meat, fish and fruit (Fig. 1a, period V) At the age of nine months when given by mistake a small piece of gluten-containing bread he again reacted with omitings, diarrhoea and severe prostration. A new si milar exposure bout two months later pro oked symptoms, but less severe From the ge f 18 months he has been gi en an ordinary diet with normal amount of gluten containing food (Fig 1a, period VI). There has been no reaction at all to this feeding. Thus there was a rapid disappearance of gluten intolerance

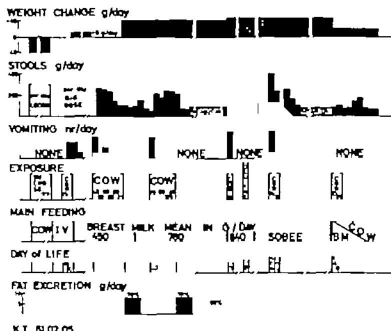


Fig. 2a. Case 2. Weight change, stool weight, fat excretion and gastrointestinal symptoms in relation to main feeding and exposures during the first hospital stay. Figures under exposure mean g/day.

Last check-up was at the age of 3½ years. His weight at this time was 19.6 kg and his height 100 cm. He was in good condition with normal physical status and no allergic manifestations.

Case 2. K. T. ♀ 610205

Summary. The patient fell ill with large, watery stools during the first few days of life. Steatorrhoea was diagnosed very early. Gluten and the disaccharides sucrose, maltose and lactose could be excluded as aetiologic factors. Several exposures to cow's milk regularly provoked gastrointestinal symptoms up to the age of 5 months when the intolerance vanished. Probably she was moderately intolerant to gluten at the age of 10 months. After the age of 15 months, however a diet with ordinary gluten content was tolerated.

Detailed history. First child of unrelated healthy parents. No siblings. Birth weight 2480 g. First exposure to cow's milk with a formula containing sucrose but no cereals, took place during the first days of life and was followed by loose stools. During day 7–17 (Fig. 2) she was fed cow's milk with added sucrose, and had big, loose stools and lost weight. Admitted 17 days old weighing 2230 g she was severely dehydrated with acidosis, hyperelectrolytaemia and haemoconcentration. Good response to iv therapy. No pathogens in stools. pH in faeces 5.8. Chloride concentration in sweat, trypsin content in faeces and pulmonary X-ray were normal. Intracutaneous test with whole cow's milk moderately positive.

After intravenous rehydration she was put on breast milk until the age of about 90 days, when the feeding was changed to a soya formula (Sobee), containing sucrose and maltose.

Breast milk as well as Sobee were well tolerated, stools were normal, there was no vomiting and she gained weight normally (Fig.).

During this period, day 19–109 she was

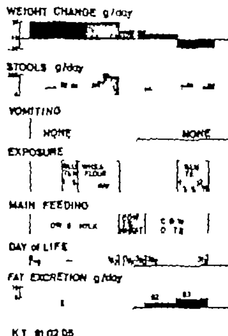


Fig. 2b. Case 2. Weight change stool weight, fat excretion and gastrointestinal symptoms in relation to main feeding and exposures during the second and third hospital stay. Figures under exposure mean g/day

exposed to small amounts of pure cow's milk five times. The exposures immediately resulted in vomiting and the subsequent passing of large watery stools (Fig. 2a). Estimation of fat absorption during two three-day periods immediately following cow's milk exposure showed moderate steatorrhoea with an absorption coefficient of about 75 (day 37–40 and 47–50 Fig. 2a). Fat absorption estimated at two occasions about 30 days after last foregoing cow's milk exposure (day 70–73 and day 136–139 Fig. 2a) was normal. It should be noted that at the time when steatorrhoea was first demonstrated, the patient had never been exposed to gluten.

The first exposure to gluten—5 g in one dose—took place at the age of 87 days—without any clinical reaction. Xylose absorption one day later was normal, (maximum serum concentration 75 mg per 100 ml; 17 excretion during 4 hours).

At the age of 140 days the patient tolerated small doses of cow's milk for the first time. During the following week she could be transferred to cow's milk as the only feeding. Stools remained normal, but there was an initial stand-still in weight.

Renewed exposure during three days to small amounts of gluten at about the age of 160 days elicited no clinical reaction, but when immediately afterwards wheat flour was included in her feeding, the amount of stools increased markedly (Fig. 2b). Fat excretion was estimated only during the first three days of this exposure and was at that time 4 g/day. The patient had to be discharged at this time—5 months old—and the subsequent evolution of the clinical state could not be checked.

Readmitted at the age of 10 months. She had been fed an ordinary diet including cow's milk with 1 % of wheat flour. An exposure to gluten during 6 days was accompanied by increasing amounts of stools, a moderate increase in observed fat excretion and weight loss (Fig. 2b). No abdominal distension. The exposure to gluten was interrupted after 6 days because of the obvious clinical deterioration. The findings are consistent with—although not definitely proving—a gluten intolerance.

Discharged at the age of 10½ months on a gluten free diet containing ordinary amounts of cow's milk. Weight 8450 g length 70 cm. After some months successively transferred to a normal gluten containing diet with no untoward effects.

At the check up at the age of 27 months she had been fed a gluten containing diet for about one year. She was in good condition, weight 11.6 kg length 91 cm.

*Case 3. M. R. ♀ 60050**

Summary. From the age of two months severe gastro-intestinal symptoms. The clinical picture suggested a malabsorption syndrome with steatorrhoea. Omission of gluten did not improve her condition. Exposure to cow's milk repeatedly provoked severe gastro-intestinal symptoms up to the age of 7 months. Disaccharides could be excluded as

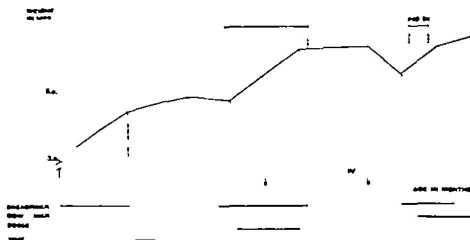


Fig. 3 Case 3. Weight increase in relation to feeding during the first year of life

a cause of her diarrhoea. Tolerance to cow's milk successively developed after the age of seven months and seemed to be complet at the age of 10 months. Exposure to pure gluten at this time caused clinical symptoms of such severity that continued exposure and balance studies were rendered impossible Tolerance to gluten appeared at bout years of age

Detailed history First child of unrelated, healthy parents. Birth weight 3300 g Breast fed two months with normal weight in

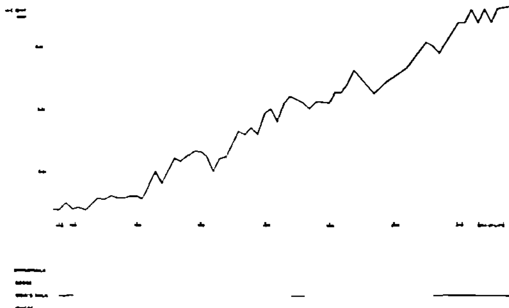


Fig. 3b. Case 3. Weight curve and main feeding during part of the first hospital stay (6–7 months of life). The two first exposures to cow milk provoked heavy vomiting (day 148–149; 184–185).

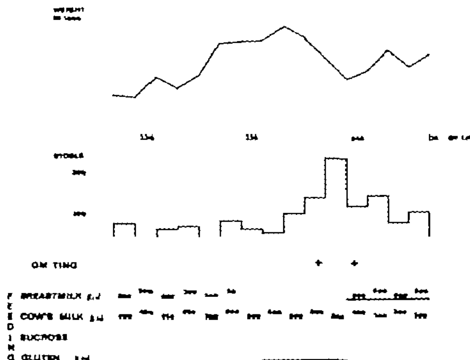


Fig. 3. Case 2. Weight curve, stool weight and gastrointestinal symptoms before, during and after a gluten tolerance test at the age of eleven months.

crease (Fig. 3a, period I). Her weight at two months of age 4430 g. She was then fed a formula containing cow's milk, sugar and wheat flour and soon started having frequent vomitings and periodical loose, foul smelling stools (Fig. 3a, period II). No improvement after omission of flour from the diet. Weight increase was poor and at the age of four months she was admitted to a local hospital.

On admission she was thin and pale with distended abdomen. Weight 4920 g. Stools typical for steatorrhoea. She was first given various formulas containing cow's milk, and disaccharides, but without cereals (Fig. 3a, period II). No improvement occurred until the age of 5 months when feeding was changed to breast milk and a soya formula (Sobee) containing sucrose and maltose. Her symptoms then successively disappeared and she began to gain weight (Fig. 3a, period III, Fig. 3b). Reexposure to pure cow's milk at the age of 6 months immediately provoked intense vomiting (Fig. 3b, day 184). By contrast wheat flour added to breast milk during a 10-day period caused no untoward effect (Fig. 3b, day 196–206). When cow's milk was added to this diet no vomitings were produced but there was a flattening of the weight curve (Fig. 3b, day 206). Discharged with a cow's milk formula containing wheat. Successively increasing vomitings. At a check up 2 months after discharge from the hospital she had not gained weight (Fig. 3a, period IV). During the following weeks receiving various formulas she demonstrated a pronounced weight lability and lost weight (Fig. 3a, period IV). The influence of the different food constituents upon her symptoms during that period cannot be evaluated.

At the age of ten months she was referred to this hospital for further investigations. She was thin and pale with reduced turgor and distended abdomen. Weight 5360 g. No rickets or anaemia. A bronchopneumonia was efficiently treated with antibiotics. Tryp-

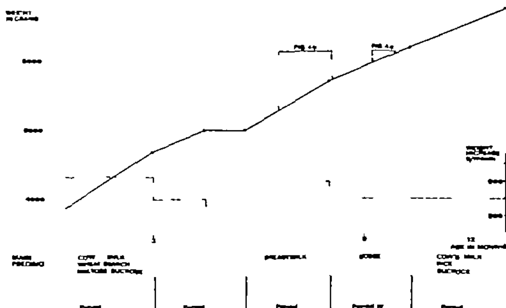


Fig 4 Case 4. Weight increase in relation t main feeding during the first year of hf

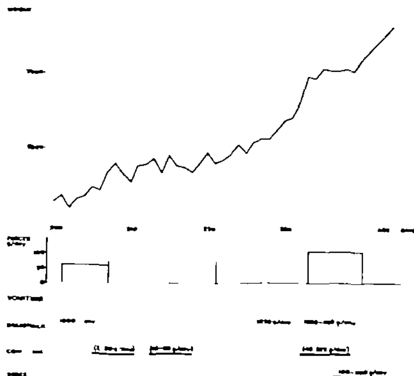


Fig. 4 b. Case 4. Weight curve day "00- 45. Main feeding breast milk. Exposed to cow milk during two periods.

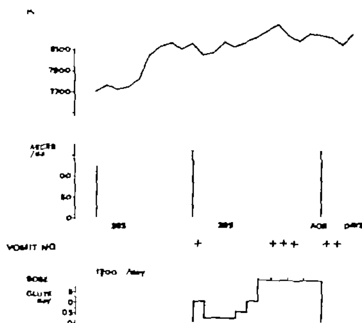


Fig. 4c. Case 4. Weight curve, stool weight and gastrointestinal symptoms before and during gluten tolerance test at the age of 9½ months. Fat excretion, see Table 1

sin content in faeces and chloride concentration in sweat were normal. Electrophoresis of serum showed somewhat low albumin (2.4 g per 100 ml). The gammaglobulin was 1.1 g per 100 ml. Intracutaneous test with whole milk and beta lacto-globuline markedly positive, with alpha lact-albumine weakly positive. After parenteral fluid therapy she was transferred to breast milk and improved rapidly. She began to gain weight and had a normal xylose and fat absorption (Fig. 3a period V). She was then given increasing amounts of cow's milk and was found to tolerate full feeding without symptoms. When 1–3 g of gluten per day was added to the cow's milk she promptly started vomiting, had loose, foul-smelling stools and lost weight (Fig. 3c). Fat absorption studies could not be performed. She was discharged on a gluten free diet containing 600–700 g of cow's milk and remained free of symptoms. Gluten in the form of wheat flour was reintroduced without symptoms at the age of 2 years. At check up one year later she was in good condition. Length was 92 cm and weight 1_.5 kg.

Case 4. M. G. ♀ 611020

Summary. Gastrointestinal symptoms from the age of three months. Admitted two months later with the clinical picture of malabsorption, which was verified by laboratory studies. There is strong evidence that her symptoms were provoked by cow's milk. Intolerance to sucrose, maltose and lactose could be ruled out. Gluten was well tolerated at this time. At the age of ten months, however, gluten intolerance causing fat and xylose malabsorption was demonstrated. At about the same time tolerance to cow's milk had appeared. Gluten was well tolerated from about the age of 22 months.

Detailed history. First child of unrelated parents. Mother eczema. Birth weight 3750 g. Breastfed 10 days and then given formulas containing cow's milk, wheat and three different sugars: sucrose, maltose and glucose. Up to the age of three months there were no feeding problems, normal gain in weight (Fig. 4a period I). A couple of weeks later she started having frequent vomitings and periodically loose stools, and her weight increase became unsatisfactory (Fig. 4

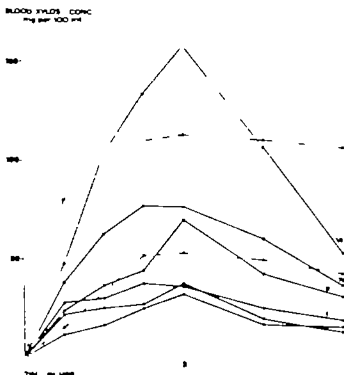


Fig 4 d. Case 4. Xylose absorption tests on different feedings. Area between broken lines normal mean value ±1.5 S.D. Curve I: Age 168 d. Cow's milk + maizena. Curve II: Age 179 d. Breast milk. Curve III Age 13 d. Breast milk + cow's milk. Curve IV Age 291 d. Sobee Curve V Age 304 d. Sobee + gluten. Curv VI: Age 332 d. Cow's milk + rice

period II). She was admitted to this hospital at the age of five months. Her limbs were thin and the abdomen distended. Weight 5980 g Moderat eczematous skin lesions. N rickets, or anaemia. Normal prothrombin time. N pathogens in stools. Chloride con centration in sweat, trypsin content in faeces and pulmonary roentgenogram normal. Intracutaneous test with cow milk was markedly positive but precipitating antibodies to cow s milk could not be demonstrated in serum.

She was first given a formula containing cow milk, maizena and sucrose but feeding difficulties continued (Fig 4a, period II). A xylose tolerance test performed during this period showed impaired absorption (Fig 4d Curve I). When put on breast milk

TABLE 1 *Case 4 Fat excretion on different feedings*

Age in day	Feeding	Fat excretion g/day
172 174	Breast milk	7
175–177	Breast milk	6
182 184	Breast milk + gluten	3
185–188	Breast milk + gluten	4,5
214–219	Breast milk + cow milk (30 g/day)	2,6
284–.88	Sobee	6*
299–302	Sobee + gluten (2 g/d.)	12,3

One week earlier the patient had been fed wheat flour during couple of days, which caused vomitings and abdominal distension.

omitings ceased ... became normal and weight increase w... ...umed (Fig. 4a, period III). Xylose ab...ption improved (Fig. 4d, Curve II). But ... absorption studies, now performed for the first time, showed slightly elevated amount of f...t in the faeces. (Table 1; day 17?–1...). At the age of six months a gluten tolerance test was performed, adding 51 g of gluten to the breast milk during a 6 day period. No symptoms appeared and fat absorption had become entirely normal (Table 1, day 182–188).

The following weeks she was exposed to small amounts of cow's milk without added sugars or cereals during two periods. Both times she stopped gaining weight, had occasional vomitings and loose stools (Fig. 4b, days (06–220; 232–239). Xylose tolerance test again showed impaired absorption (Fig. 4d, Curve III), but fat excretion was normal (Table 1, day 214–19). Now, at the age of 8 months, she was transferred to a soya formula (Sobee), which was well tolerated (Fig. 4a, period IV). Xylose absorption was normal (Fig. 4d, Curve IV).

When at the age of nine months wheat was added to the Sobee formula she reacted promptly with heavy vomitings and abdominal distension. One month later a new gluten tolerance test was done. A single dose of 1 g of gluten immediately provoked severe vomiting and prostration. With a dose of 0.5 g ...4 times a day symptoms were limited to small vomitings, loose, foul-smelling stools and weight stand-still (Fig. 4...). Impaired fat and xylose absorption could be demonstrated (Table 1, day ?98–302, Fig. 4d, Curve V). When she was fed Sobee with rice flour she soon recovered. During the following weeks cow's milk was given in increasing amounts without causing gastrointestinal upset or impairment of xylose absorption (Fig. 4d, Curve VI). From the age of 10 months she was fed a gluten free diet containing 500–600 g of cow's milk and thrived well (Fig. 4a, period V). From the age of ?2 months wheat flour has been added to he... feeding with no obvious untoward effect. At the age of ?7 months her length was 85 cm and her weight 12.6 kg.

Comments and Discussion

These four patients demonstrate a similar clinical picture with the following main features: 1) Early feeding of cow's milk. 2) Onset of gastrointestinal symptoms during first few months of life with vomitings and frequent, big, often watery stools. Malnutrition and laboratory evidence of a malabsorption syndrome. 3) Rapid improvement on a diet not containing cow's milk; immediate deterioration on reexposure. 4) Tolerance to cow's milk appears successively. 5) Gluten—initially well tolerated—later provokes symptoms. 6) Gluten tolerance appears at about the age of two years.

Mucoviscidosis and chronic intestinal infection have been excluded in all cases. Other rare causes of steatorrhoea such as beta globulin deficiency [29] or intestinal malformation have not been ruled out systematically but are not likely. Lately it has been shown that disaccharides and monosaccharides may provoke chronic diarrhoeal disease probably because of specific enzymatic deficiencies [8, 10, 11, 21, ..5, 34]. This aetiology was excluded in our patients since breast milk and Sobee containing the relevant sugars were well tolerated at the same time as cow's milk provoked severe symptoms. Moreover vomiting—an outstanding feature of our patients—is not a typical symptom at least not in disaccharide intolerance.

The initial intolerance to cow's milk has in all our cases been convincingly proved by repeated exposures. Unfortunately sufficient amounts of purified proteinfractions were not available for a more close definition of the aetiologic factor. In three cases the malabsorption was de-

monstrated in this hospital by means of fat absorption determinations and/or xylose tolerance tests. In the fourth patient increased fat excretion during the first stage of the disease was suggested by the typical appearance of the stools and by their high fat content—55 % of dry weight It can be argued that with the presenting symptoms that is vomitings and watery stools, balance studies would be technically difficult and unreliable and that a rapid bowel passage per se could give n impaired absorption But in the presented cases these symptoms promptly disappeared when the offending food was excluded, and it was possible to demonstrate a malabsorption even after cessation of the acute symptoms. In this connection it may also be mentioned that villous atrophy disappearing after the omission of cow's milk recently has been observed in two adult patients with steatorrhoea [31 33].

The relation to classic gluten induced coeliac disease is complex. While gluten intolerance was not evident in the beginning of the disease it appeared later clearly demonstrated in three of the cases and likely in the fourth. Compared with the coeliac disease the gluten induced malabsorption in our cases was moderate This might have been due to short exposure and to the fact that relatively small amounts of fat were contained in the food given during the balance periods. Another feature of our cases was the transient nature of the gluten intolerance. This reminds of the observations made by SHELDON *et al* [3_] and by CHAPTAL *et al* [6], who demonstrated that the duration of the gluten intolerance was very long (lifelong!) in some patients but of short duration in others. The latter cases are in this respect similar to ours. The rapid clinical improvement in our patients after omission of the offending food both during the cow's milk sensitive period and the gluten sensitive period, talks against an intestinal damage as profound as that observed in classic gluten intolerance [_].

The main point made in our communication is the sequence of events with a primary cow's milk intolerance and a later developing gluten sensitivity HEINER *et al* [19] in 196_ on the basis of antibody studies—and brief mentioning of clinical observations—advances the hypothesis that in certain patients an early cow's milk intolerance may set the stage for" a later developing gluten sensitivity The results of our study seems to be a good illustration to the validity of this hypothesis.

Regarding the pathogenesis in classic gluten provoked steatorrhoea a toxic action of gluten and an allergic mechanism have been discussed [3 13 18 33 4 19]. The same possibilities should be present in our cases The appearance of sensitivity to two different food constituents—probably proteins—could speak in favour of an allergic mechanism. Furthermore the immediate symptoms induced by cow's milk as well as by gluten are very similar to an anaphylactic reaction, and may also speak in the same direction. The relevance of the circulating antibodies demonstrated is hard to evaluate since such antibodies often appear in the serum of normal babies fed cow's milk [17]. Probably the strong skin reactions observed in two of our patients have greater significance in the diagnosis of cow milk allergy as pointed out by GOLDMAN *et al* [15] Whatever the pathogenetic mechanism in these cases

the change from [illegible] milk intolerance to a gluten sen[illegible]ity is of particular theoretical int[illegible]est when discussing the aetiology and pathogenesis of coeliac di sease

Summary

Four infants with a malabsorption syndrome are [illegible]escribed. The clinical picture was typical and can be summarized as follow[illegible] 1) Early feeding of cow's milk, 2) Early onset of gastrointestinal symptoms with vomitings and frequent large watery [illegible]tools. Malnutrition and laboratory e[illegible]idence of a malabsorption syndrome 3) Rapid improvement on a diet not containing cow's milk, immediate deterioration on reexposure to cow's milk 4) Tolerance to cow's milk appears successively 5) Gluten—initially well tolerated—later provokes symptoms, 6) Gluten tolerance appears at about the age of two years.

Exposures and balance studies unequivocally establish an initial cow's milk intolerance later changing to a gluten sensitivity both leading to a malabsorption syndrome

The findings are discussed with regard to current theories concerning the pathogenesis of gluten induced coeliac disease

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Department of Paediatrics
Barnsjukhuset
Göteborg
Sweden

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From the Depart   ts of Paediatrics and Pathology I, University of Uppsala, and the Departme   t of Medical Biochemistry Uni ersity of Gothenburg Sweden

Congenital Amaurotic Idiocy

by BENGT HAGBERG GÖSTA HULTQUIST ROLF ÖHMAN and LARS SVENNERHOLM

Infants with amaurotic idiocy of the Tay-Sachs type usually develop quite normally up to the age of 4–6 months or later A very rare congenital form of familial cerebral lipidosis resembling Tay Sachs disease has been described by Norman & Wood [7] and Brown *et al* [2] in two non-Jewish siblings As far as we know no other comparable cases have been reported. The present paper concerns a Swedish case which had its clinical onset late in the newborn period and where chemical investigations revealed the presence of an abnormal ganglioside pattern.

A. Clinical picture

Case S 4

No. 151/62 U-a —The boy was born on October 22rd 1960 and was the second of three children in a non-Jewish family without known consanguinity Both parents were deaf the mother (b 1936) possibly after a complicated whooping cough in infancy the f ther (b 1929) due to a congenital form. His deafness might have been related to a severe eclampsia from which his mother died at deli ery Our patient s elder sister (b 1958) had died at the age of 5 months when operated for a malformed double aorta. His younger brother (b 1963) was found to be healthy at followup examination when 1 year old. The mother s pregnancy was uneventful. The patient was born at term after a normal delivery and with a birth weight of 3910 g At first he was considered to be quite normal and was easily nursed at the breast

When the boy was two weeks old he developed a series of clonic fits, some of them focal, some generalized. H was sent to the local paediatric department where he was found to be in a poor condition with cyanosis and head retraction but without any laboratory signs of meningitis Pyridoxine was given intramuscularly without effect and medication with various antiepileptics which was tried during the next days was no better His fits increased in frequency and intensity episodes of vomiting and respiratory distress were soon added, and the boy now lay in a st te of continuous, pronounced rigidity He had to be fed by tube When 5 weeks old the convulsions had diminished but he now seemed to be blind with poorly reacting pupils, had continuous nystagmus, and it was no longer possible to establish any sort of contact His cerebrospinal fluid protein level was now found to be 460 mg per 100 ml. His optic discs which at first examination had only revealed engorged veins slight oedema and minor haemorrhages, had now developed severe changes protrudent and pigmented macular patches and incipient bilateral optic trophy These changes progressively increased during the next few months, but otherwise the patient seemed to

be in more stationary and burnt out stage of decerebrat rigidity reacting to sounds but not t light. Among the laboratory tests, his toxoplasma titres in particular were fol lowed but were repeatedly found to be nega tive. This was also the case with his mother The Wasserman test was negative X ray f the skull did not show any intracranial calci fications.

When 13 months of age the boy was examined at the Paediatric Department University Hospital, Uppsala. At that time he was still in a state of severe muscular rigidity had muscular contractures and se condary hip dislocation on the right side. H still had repeated clonic fits. The head circumference was 44 cm. No malformations were found. *EEG showed lack of normal* basal rhythmic activity and there were no bvious signs of any cortical activity The cerebrospinal fluid revealed normal number of cells and a protein level of 51.3 mg per 100 ml. The electrophoretic pattern showed low $alpha_2$- and beta-globulins but was otherwise normal. Ventriculography re ealed enormously dilated ventricles and the walls of the cerebral hemispheres were paper thin. The cerebellum seemed to be under developed. Routine haematological and uri nary tests were normal.

The optic discs showed marked trophy and pigmented chorioretinal protrusions.

Due t these changes and the progressive neurological deterioration an typical sort of amaurotic idiocy was suspected. As no treatment was considered possible and the boy was severely retarded he was transferred t an institution for feeble minded and stayed there until he died from bronchopneumonia at 19 months of age.

Special investigations

Vacuolated lymphocytes were looked for in the peripheral blood but none was found. The bone marrow was also examined and found t be normal. The urine was tested for gangliosides but none was found. Toluidine blue stained urinary sediments were repeatedly studied for metachromatic substances and occasionally large, redbrown mulberry shaped bodies were present The sulphatide content was 1.5 μg/mg creatinine (normal < 5μg). Abnormal aminoacids or reducing substances were not present in the urine *Rectal biopsy* as described by Nakai & Landing [6] was performed twice and changes very similar to those in Tay-Sachs disease were found (see below). *Cerebral biopsy* was performed from the occipital lobe and supported the diagnosis of an advanced stage of cerebral degeneration suggesting some sort f amaurotic idiocy (see below)

B Post mortem findings

Methods

Biopsy *material from the rectal mucosa* was fixed in 10 formalin and in Zenker solution. Biopsy material from the brain was fixed in formalin.

The autopsy was performed in county hospital and due t technical reasons all the brain material was immediately deep frozen and only later were the specimens fixed in 10 % formalin. From all other organs the material was immediately placed in 10 formalin.

The staining methods used were: haematoxylin and eosin, van Gieson, PAS, Sudan black, Scharlach R, Mallory' phosphotungstic acid haematoxylin, Alcian blue Holzer glial fibre method, Nissl ganglion cell method, von Kóssa method for calcium deposits, cresylviolet-acetic acid, Baker acid haematein test with pyridine pretreatment and Heidenhain method for myelin sheath.

Results

Biopsy material was obtained when the boy was 14 months old

Brain tissue and meninges. The brain tissue is composed of glial elements with enclosed granular deposits. The glial tissue is rather rich in cells with partly elongated, bipolar partly rounded nuclei with a moderate content of chromatin.



Fig. 1 Brain biopsy with gliosis and f t deposits partly around vessels. Frozen section. Sudan black. 560

The gliosis is mainly the result of an increase of microglial cells but there is also some increase in macroglial elements. Holzer-stained sections also show a moderate amount of glial fibres The sclerotic glia is divided into compartments which contain abundant granular structures The granular bodies sometimes have a brighter central zone and a denser body at the periphery which gives the impression of some sort of ganglion structure No typical ganglion cells have been found in the cerebral biopsy material. In some places the granular bodies have a concentric lamellar structure With various staining methods the bodies in some preparations look like calcium granules but the staining reaction for calcium did not give any unequivocally positive result Sudan black and Scharlach R show a moderately positive reaction in the granular material and the surrounding glia The granular masses show strong anisotropism Cresyl violet staining is negative while the PAS-staining is rather strongly positive Acid haematein test is also strongly positive. The staining for myelin sheath is inconclusive but the granular material gives a positive staining reaction with the Heidenhain method. The meninges reveal no abnormalities.

Rectum. Some ganglion cells of the myenteric plexus show vacuolisation and very slight ballooning of the cytoplasm PAS-staining is negative in formalin fixed material and weakly positive after Zenker fixation. The ganglion cells do not contain any sudanophilic material

Necropsy findings Autopsy was performed three hours after death. When the skull was opened the cerebral hemispheres could be seen pressed against the *dura mater* The brain was like a fluctuating membrane and it was not possible to detect any distinct sulci or gyri. When the



Fig. 2. Brain biopsy with PAS positive deposits in the brain tissue partly around vessel. PAS stain. 715

brain was taken out the very thin cerebral membrane ruptured and 700 ml of straw coloured fluid poured out The cerebral hemispheres were reduced to paper thin (1–2 mm thick) shells There was a marked atrophy of the cerebellum affecting mainly the hemispheres but also the vermis. The brain stem appeared normal. The total weight of the brain was 130 g

As the cerebrum was now like a col-

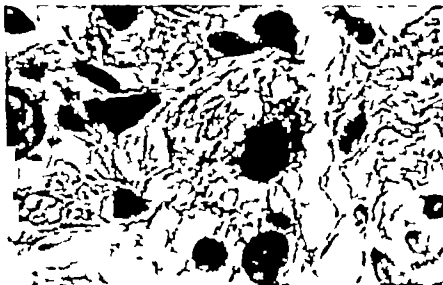


Fig. 3. Rectal biopsy with vacuolated ganglion cell. Gierson stain. 1790



Fig. 4. A sagittal section through an eye with spot of pigment atrophy in the fundus.

lapsed cyst and it was impossible to distinguish different anatomical areas all the brain material was immediately deep-frozen From the frozen material small samples were cut out and placed in 10 % formalin

Eyes. In the fundus there was an irregular area lacking pigment and with a whitish yellow colour No protruding pigmented masses were seen in the autopsy specimens

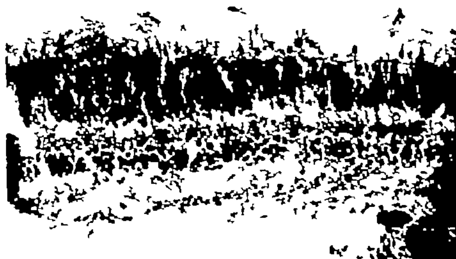
Microscopical examination Cerebral hemispheres The changes are essentially similar to those seen in the biopsy material.

Cerebellum and brain stem show some atrophy Artefacts from ice crystals prevent detailed histological examination The molecular layer shows some atrophy There is no evident loss of Purkinje cells and they seem to be of normal form and shape In some cells the nuclei are slightly shrunken. There is no evidence for any PAS-positive material in the nerve cells. Sudan black stains the Purkinje cells stronger than normally

Peripheral nerves are apparently normal.

Eyes In the macroscopically altered area of the retina there are distinct changes in the various cell layers. The pigmented epithelium is atrophic and in some places difficult to detect. In the rod and cone layers the rods are rather prominent in Sudan black stain but appear to be diminished in number There seems to be a more marked loss of cones. Both the nuclear layers show a certain atrophy especially the inner one which by Sudan black staining shows separate blackened cells but in Scharlach R staining shows fewer such cells. By the Sudan black staining method the plexiform layers appear very scanty in comparison with the control material and in the ganglion cell layer only a single minute ganglion cell is seen In these layers some fattened cells glial elements or perhaps other fat phagocytising elements, are demonstrated No other important changes are seen

Spleen The follicles are large with a





b

Fig. 5. a. Atrophic retina b. Retina from case of about the same age. Frozen section. Sudan black ×0

moderately cell rich marrow and with many cells of reticuloendothelial origin Some cells with large ballooned nuclei in their cytoplasm contain coarse-grained material which is positive to Sudan black. Sometimes these cells appear in small groups.

L ve The Kupffer cells are prominent

TABLE 1 *Lipid composition of brain tissue*

The values are expressed as per cent of fresh weight, except for cerebellum where per cent of dry tissue is used.

	Case S.A. Cerebrum (total)	Grey matter I	Grey matter II	Grey matter III	White matter I	White matter II	White matter III	Case S.A. Cerebellum (total)
		NORMALS (frontal lobe)						
Water	91.4	87	87.2	82.3	81.6	[illegible]	1.9	79.5*
Nitrogen	—	1.38	1.17	1.89	1.37	1.63	1.92	11.4
Hexosamine (total)	—	0.09	0.11	0.14	0.08	0.10	0.10	0.21
Total lipids	1.80	3.86	4.12	6.16	[illegible]	13.08	15.[illegible]	59.5
Cholesterol	0.52	0.76	1.01	1.15	2.24	3.30	4.3*	6.6
Phospholipids	1.90	3.00	3.04	3.89	5.00	6.75	6	14.4
Cephalins	0.45	1.33	1.49	1.96	[illegible]	3.44	4.41	6.6
Lecithins	0.34	1.35	1.33	1.68	1.69	[illegible]	[illegible]	6.1
Sphingomyelins	0.10	0.27	[illegible]	[illegible]	0.45	0.83	1.18	1.4
Cerebroside and sulphatid hexose	0.02	0.02	0.01	0.03	0.43	0.82	[illegible]	0.24
Gangliosides								
Lipid hexosamine	0.006	0.021	0.024	0.031	0.013	0.014	0.014	[illegible]
Lipid N-acetyl neuraminic acid	0.011	0.060	0.072	0.067	0.030	0.029	0.032	[illegible]

I = 13 months, II = 19 months, III = 8 months, S.A. = 10 months.
* Percentage of the fresh weight.

Sweden) was rinsed with large volumes of water and n-propanol before use. Thin layer chromatography was performed on plates with Silica Gel G (Merck, AG, Darmstadt). The plates were prepared with a commercial apparatus (DESAGA, [illegible] 600, C. Desaga GmbH, Heidelberg, Germany).

Results and Discussion of Quantitative Determinations

The results of the quantitative determinations are given in Table 1 and 2. The lipid content was reduced in the brain tissue. In the cerebral hemispheres there were very low amounts of typical myelin lipids, e.g. cerebrosides, which agrees well with the morphological picture. The concentration of the other lipids was also very low or only about 1/3 of the amount in normal cerebral grey matter. However the concentration of cholesterol was about normal. It is particularly interesting that the concentration of gangliosides was lowered to about the same or a greater degree than the other lipids. This is in sharp contrast to the microscopical findings suggesting an increase in gangliosides. In the cerebellum the total lipid content was also lower than normal. The reduction mainly affected glycerophospholipids and cerebrosides. Only trace amounts of triglycerides were present. Significant amounts of cholesterol esters were found in neural tissue from our case, somewhat less in the cerebellum than in the cerebrum. The histochemical investigation showed a moderately positive Scharlach R reaction in the granular material and surrounding glia. The occurrence of cholesterol esters is an abnormal finding suggesting a disturbed lipid metabolism.

TABLE 2. *Lipid composition of extra-cerebral tissues*

The values are expressed as per cent of dry weight.

	Liver	Spleen	Kidney
Water*	77.2	78 6	83.7
Nitrogen	1_1	12.1	12.3
Total lipids	7.2	7.5	7 1
Cholesterol	2.1	1.9	1.8
Phospholipids	5.0	5.4	5.0
Cephalins	1.3	1.8	2.0
Lecithins	3.8	2.4	2.0
Sphingomyelins	0.0	1 1	0.8
Glycolipid hexose	0.14	0.21	0 25

* Percentage of fresh weight.

Other parenchymatous organs as spleen liver and kidney only showed slightly reduced lipid values The cholesterol con tent was normal or possibly somewhat high, while phospholipids were reduced.

In the Bristol case [ ] only cholesterol and total phospholipids were assayed in brain tissue The phospholipid content was low as also found in our case However the cholesterol content was increased about threefold

The Bristol case [2] was called a cerebral l pidosis. From a biochemical point of view t is evident that this case has nothing in common with the true neurolipidoses which are all known as disturbances of sphingolipid metabolism

Isolation and Characterisation f Gangliosides

The bram material was homogenized in a Turmix blendor during the addition of a total of 7 vol. chloroform-methanol 1 1 v/v After beating t boiling point the extract was filtered and the residue w re-extracted with 5 vol. chloroform-methanol 2 1 v/v Finally the residue was extracted with chloroform methanol 1 2, v/v in Soxhlet apparatus. The extract were pooled together and taken to dryness in a rotating evapora tor The lipids were then re-extracted with chloroform-methanol 2 1 v/v

The gangliosides were separated from other lipid by chromatography on cellulose A column was prepared from 50 g cellulose powder slurried in the lower phase f chloroform methanol water (C-M W) 16 4 1 v/v The lipids were dissolved into 4 ml of the same solvent and put on the column. The chromatographic procedure was a discont nuous gradient elution with 10 vol. C M W 16 4 1 v/v (A) 10 vol. C-M W 3 15 1 v/v (B) and 6 vol M-W 9 1 v/v (C) The eluates were tested by thin layer chromatography on silica gel in chloroform-methanol water and n-propanol water systems using am monium molybdate-perchloric acid or resor cinol-hydrochloric acid spray reagent for detection.

The major amount of resorcinol positive substances was found in eluate B while small amounts were present in eluate C In fraction A, at least three sialic acid positive spots could be indicated. Two of them had R_f-values very close to that of the monosialoganglioside G_{M3}, isolated from spleen [1] The third one was moving still faster in the propanol water solvent However fraction A contained the bulk of the lipids other than gangliosides and was not further investigated.

The luates B and C were dialysed against distilled water taken to dryness with a rotating evaporator and redissolved in chloroform-methanol 1 v/v Fractions B and C were pooled together and regarded as crude ganglioside fraction.

When the ganglioside pattern of normal child brain was compared with that of the present case it was evident that the latter contained large amount of sialic acid

positive compound which moved between normal brain mono- and disialogangliosides on thin layer chromatograms. It may be noted that the monosialoganglioside G_{M2} which accumulates in the brain of cases with infantile familial amaurotic idiocy did not occur in increased concentrations in the present case

The crude ganglioside fraction was now further separated on the paper roll column in a propanol water system. The column was first developed with a mixture of a propanol water (P–W) 80 20 v/v The gangliosides were then dissolved in 1 ml P–W 75 25 v/v and put on to the column. The column was then run with 250 ml P–W 72 25 v/v 150 ml P–W 70 30 v/v and 200 ml P–W 50 50 v/v The effluent was collected on an automatic fraction collector with about 10 ml in each fraction. The eluates were assayed for sialic acid using the resorcinol hydrochloric acid method and investigated by thin layer chromatography on silica gel.

The fractions containing the unidentified substance were pooled together This preparation was further shown to contain small amounts of other monosialogangliosides (G_{M1} and G_{M3}) and traces of disialoganglioside G_{D1a} [13] The new compound was homogenous in different solvent systems for thin layer chromatography

An attempt was made to isolate the compound in pure form by means of preparative thin layer chromatography After visualization of the substance by spraying the plate with water the spot was scraped off The compound was extracted from the silicic acid with chloroform methanol 1 * v/v The extract was filtered and taken to dryness with a rotating evaporator The substance was then taken up in chloroform methanol _ 1 Analytical thin layer chromatograms revealed however that an extensive degradation had occurred. As there were only small amounts of the new compound no further attempt was made to isolate the substance in pure form.

Enzymic hydrolysis of the new compound with neuraminidase (RDE) was therefore performed on the incompletely purified fraction. Samples containing approximately 10 μg sialic acid were incubated at 37 C for 30 minutes to 48 hours in a sodium acetate-acetic acid buffer pH 6 [1] containing 1 IU RDE in about 30 μl. The enzymic hydrolysis was studied by thin layer chromatography Resorcinol-hydrochloric acid and ammonium molybdate-perchloric acid spray reagents were used for the detection The reference substances used were glucose galactose N acetyl neuraminic acid, different monosialogangliosides and neutral glycolipids prepared by acid hydrolysis of normal brain monosialoganglioside

A fast hydrolysis of the unknown compound occurred. As shown in Fig 8 free sialic acid and two new sialic acid-containing lipids were formed. The faster moving of these closely moving lipids was chromatographically identical with monosialoganglioside G_{M3}

At the same time as the decrease in gangliosides there appeared a new compound which moved in two different solvents with the same R_F-value as ceramide-lactose prepared by acid hydrolysis of normal brain monosialoganglioside

Impurities of normal monosialogangliosides in the substrate were not affected during treatment with RDE while the trace amounts of disialogangliosides had completely disappeared.

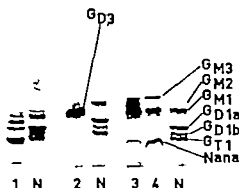
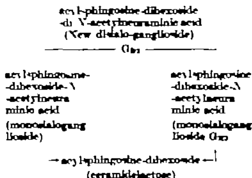


Fig. 8. Thin layer chromatograms of brain gangliosides, developed in propanol water 70:30, v/v for 3 hrs. Spray reagent: resorcinol HCl. N—Ganglioside pattern of normal human brain tissue. 1—Ganglioside pattern of brain tissue in present case. 2—"New" ganglioside purified. 3—Sample of "new" ganglioside containing 7 μg of N-acetylneuraminic acid, incubated with 1 IU neuraminidase for 20 minutes at 37°C. 4—The same components incubated for 2 hrs at 37°C.
The different gangliosides are designated as previously suggested [13]. Consequently the new ganglioside is called G_{D3}. Nana stands for N-acetylneuraminic acid.

Discussion of Ganglioside Structure

The results support the hypothesis that the investigated compound is a disialoganglioside with a carbohydrate moiety of two hexoses. As in the lactose-monosialoganglioside isolated from spleen (G_{M3}) one of the sialic acid molecules is supposed to be bound to C–3 of galactose which is supported by the finding of the same R_F-value for the partially hydrolyzed new ganglioside and G_{M3} at thin layer chromatography. The attachment of the second sialic acid is quite unknown.

The enzymic hydrolysis of the new disialoganglioside can be schematically represented in the following manner:



In liver and spleen ganglioside preparations a disialoganglioside with the same chromatographic characteristics as the presumed disialoganglioside from the present case has been found [8]. Work is now in progress to isolate this disialoganglioside in pure form from spleen and liver. In normal cerebral white matter small amounts of a ganglioside with the same chromatographic mobility as the new ganglioside can also be seen.

D. General discussion

The clinical picture of epilepsy, idiocy, blindness and pronounced degenerative changes in the optic discs relate this case to the amaurotic idiocies. This classification was further supported during life by the histological findings at rectal and brain biopsies. The clinical subgroup to which this case should be referred is more difficult to find. There are similarities with two siblings from Bristol described as cases of congenital amaurotic idiocy [–, 7] but important differences also exist. The clinical onset was at two weeks in our case, while the Bristol cases were already in poor condition a few hours after birth. Convulsions were a dominating symptom in our case but were of secondary im

portance in the English ones The ophtalmoscopic appearance of the optic discs also differed At autopsy a small, shrunken and very firm brain was found in the two Bristol cases while our case had large brain cavities nearly devoid of brain parenchyma in the thin walls.

The microscopical picture also had some similarities with that of the two siblings from Bristol However in the cerebral hemispheres of our case there were no longer any cells recognizable as neurons but spherical granular masses containing numerous nuclear remnants were found The glial cells were only moderately increased but in the English cases there was an enormous number of cells These could not be distinctly identified but were probably of glial origin. In the cerebellum slight atrophy and histological changes were revealed in our case while marked atrophy and cell degeneration characterized the Bristol cases The general morphological picture in the two English cases gives the impression of a pronounced immaturity of the brain tissue This may well be the case also in our patient but the destruction of brain tissue was too large for definite conclusions.

The main chemical characteristic was a tissue poor in typical brain lipids. The lipid pattern was more like that found in young foetal brain or in a supposed intermediary between an extracerebral parenchymatous organ and a developed brain — Neural tissue has a much higher concentration of gangliosides than any other organ. The pattern of brain gangliosides is also specific In nerve tissue the predominant gangliosides are composed of a tetrasaccharide bound to acylsphingosine containing one to three molecules of sialic acid [4] In other parenchymatous organs the major gangliosides are composed of acylsphingosine bound to a disaccharide (lactose) containing one or two sialic acids [8 14]. In the present case the concentration of brain gangliosides was much lower than in normal children The ganglioside pattern was composed of ordinary brain gangliosides and gangliosides of the extracerebral type These findings may be considered a biochemical indication of a defective differentiation of the brain tissue in the present case

The biochemical results are in contrast to the morphological and histochemical findings from which there was a probable increase of gangliosides and other lipids The situation is not unique In all forms of amaurotic idiocy there are histochemical signs of an increase of gangliosides which have never been verified with chemical methods, except in the infantile form (Tay-Sachs) In our opinion it is important to realize that the tinctorial properties of a chemical compound is much more dependent on the physico-chemical state than on the concentration In the present case the physico-chemical conditions must be profoundly changed from the normal

Histochemically an abnormal increase of lipids in the sections was found to be decidedly focal and perivascular while other parts were quite negative to lipid stains Thus the focal accumulation of lipids does not necessarily contradict the chemical results

In conclusion we would like to classify our case clinically and histologically as a form of amaurotic idiocy We suggest that it is also best termed congenital amaurotic idiocy although it showed distinct differ

ences from the two Bristol siblings and is probably determined by another recessive gene. The chemical results show that our case cannot be classified as a true lipidosis. This fact however does not contradict a classification among the amaurotic idiocies which is a heterogenous group of disorders most of which do not belong to the lipidoses. Hitherto among the different amaurotic idiocies, only the infantile amaurotic idiocy of T y-Sachs has with certainty been shown to be a true lipidosis.

Summary

A combined clinical histological and biochemical report is given of a 10-months-old boy who died after a progressive neurological disorder starting before or at two weeks of age and presenting a clinical picture of amaurotic idiocy. At autopsy large brain cavities nearly devoid of cortical brain parenchyma were revealed. The microscopical picture of the cerebral hemispheres showed no cells recognizable as neurons, but spherical granular masses. The histochemical findings supported the presence of increased amounts of gangliosides and other lipids. However the main chemical characteristic was a tissue poor in typical brain lipids. The ganglioside pattern was composed of ordinary brain gangliosides and gangliosides of an extra cerebral type. Further studies of isolated gangliosides suggest that a major compound is a disialoganglioside with a carbohydrate moiety of two hexoses. The observed discrepancies between the results of the histochemical and the chemical investigation of brain tissue are discussed.

It is suggested that the present case is best grouped as a congenital amaurotic idiocy but belonging to a different type from that earlier described. In contrast to T y-Sachs infantile type the new disorder cannot be classified as a true lipidosis.

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(B.H.) Dept. of Paediatrics
University Hosp.
Uppsala
(G.H.) Dept. of Pathology I
Uppsala
(R.Ö. and L.S.) Dept. of Med. Biochemistry
Gothenburg
Sweden

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From the Surgical Department of the Pediatric Clinic, Karolinska Sjukhuset
Stockholm, Sweden

Cutaneous Uretero-Ileostomy in Children

by ALEXANDER LIVADITIS

In certain urological disorders of childhood urinary diversion becomes necessary Uretero sigmoidostomy has proved to be unsatisfactory du to well-known late complications. Moreover the vast majority of children requiring urinary diversion have a defective anal sphincter mechanism that makes ureterocolonic implantation useless. These problems have been largely solved by the procedure of cutaneous uretero-ileostomy described by Bricker [5] n 1950

This however is not to imply that the latter method s without hazard or complications The aim of this study is to evaluat the efficacy of the procedure in particular respect to the operativ complications, mortality and late functional results. For this purpose pediatric patients seem more suitable than adults where the results are complicated by factors related t the primary disease 1) the operation is frequently performed in conjunction with another major procedure (e g pelvic evisceration or cystectomy); 2) the postoperative course is often complicated by continuation of the primary disease (cancer); and 3) the morbidity i increased due t preoperative or postoperative irradiation.

Material

Cutaneous uretero-ileostomy has been performed in 25 children, 6 males and 19 females, admitted to the Surgical Department of the Pediatric Clinic Karolinska Sjukhuset during the last six years. Age at operation ranged from 21 months to 18 years

Primary lesions

The primary lesions are summarized in Table 1 Nineteen patients presented themselves with neurogenic bladders due to congenital myelodysplasia or acquired disorders. Exstrophy f the bladder was the primary lesion in 5 patients, 2 belonging t our own series of exstrophy and represent those who did not achieve continence following primary bladder reconstruction. Three had previously been operated on in other hospitals. One patient had a rhabdomyosarcoma of the bladder

Symptoms

Urinary incontinence was the commonest symptom, found in 23 patients. Recurrent urinary tract infection was present in 19 patients. Fecal incontinence was encountered in the majority of the patients with spinal lesions. This, however was minor problem when compared to urinary incontinence and was usually satisfactorily managed by conservative measures. Two patient with exstrophy of the bladder operated on elsewhere with ureterocolonic diversion had a weak anal sphincter This resulted in a

TABLE 1 *Cutaneous uretero-ileostomy Primary lesions (*5 patients)*

Neurogenic bladder	19
Exstrophy of the bladder	5
Bladder tumor	1

permanent leak due to the inability of the sphincter to cope with fluid feces. Twelve patients were paraplegic and had received orthopedic treatment in an effort to render them ambulatory Anemia, fatigue dizziness, nausea and vomiting were present in 8 patients Recurrent macroscopic hematuria was present in the patient with the rhabdomyosarcoma of the bladder

Indications

Operative indications are summarized in Table 2 In 12 patients marked renal impairment with far advanced hydronephrosis made urinary diversion a matter of necessity In patients the procedure was performed in an attempt to salvage kidneys damaged by pyelonephritis after ureterocolonic implantation. Urinary incontinence in 10 children was such a distressing problem that operation was judged necessary In one patient with bladder malignancy the procedure was performed in conjunction with cystectomy

Preoperative preparation and operative technique

Preparation

The proposed operation and consequent necessity of wearing a receptacle postoperatively are explained to the parents. We found it valuable to bring the parents of the operative candidate in contact with parents whose children had undergone this type of operation. This measure decreases apprehension and has had a persuasive effect on parental assent to surgery

The site of the ileostomy is predetermined as usual. Deformities associated with cord

TABLE 2 *Cutaneous uretero-ileostomy Indications (25 patients)*

Advanced hydronephrosis and deterioration of renal function	11
Ascending pyelonephritis and renal failure following colonic implantation	3
Intractable urinary incontinence	10
Rhabdomyosarcoma of the bladder	1

lesions may cause variations in site The presence of scoliosis or lordosis as well as the possible need for an orthopedic corset must be considered.

Operative technique

The operative technique used is essentially the same as employed *originally* by Bricker in adults The operation in children is usually performed for non malignant lesions. This implies that the whole future of the child largely depends upon the degree of technical accuracy and perfection

The abdomen is entered through a left paramedian incision to avoid interference with the site of the ileostomy The appendix is routinely removed A distal ileal segment is isolated. We feel this should be as short as possible (10–12 cm) to ensure prompt emptying This precaution is imperative in children in whom further increase in bowel size is anticipated. The continuity of the remaining bowel is restored by conventional end to-end anastomosis The left ureter is passed through the root of the sigmoid mesentery Implantation of the ureters into the isolated ileal segment is then performed by use of interrupted sutures in two layers The ileal bladder is placed intraperitoneally behind the mesentery with the left end attached to the posterior peritoneum The ileostomy is constructed by circular excision of the abdominal wall, suturing the everted bowel to the skin with cat-gut The ileal bladder is positioned anterior or posterior to the cecum, depending upon the individual topographic conditions.

Postoperative complications and results

The postoperative complications are presented in Table 3

A 2½ year old girl died in the immediat postoperative period. In this instance opera tion had been proposed two years earlier but was rejected by the mother With deter ioration of renal function operative interven tion later became imperative In spit of apparently normal serum albumin and electrolyte values dehiscence of the abdominal wound and the uretero-ileal anastomosis occurred. Ureteral re-implantation was done but the patient died two weeks after the initial procedure. Autopsy revealed acut and chronic pyelonephritis and peritonitis.

Intestinal obstruction occurred in the immediate postoperative period in two children requiring a secondary laparotomy In one case this was due to herniation of the small bowel adjacent to the conduit, in the other to intraperitoneal adhesions. Fibrous adhesions caused obstruction in one child 3 years after the original procedure. This patient subsequently developed a recurrent stomal stenosis necessitating reconstruction. Stomal stenosis in another child occurred one year after operation, causing recurrent hydronephrosis. Prolapse of the ileostomy occurred in 2 patients. One required revi sion while the other was successfully treated by manipulative reduction. Super ficial stomal bleeding occurred in 4 pa tients. Five patients had minor problems connected with the ileal stoma (dermatitis crystal deposition squamous metaplasia) or the maintenance of a water tight fit of the appliance Three girls had a purulent discharge from the retained urinary bladder This condition was satisfactorily managed by periodic bladder irrigations.

TABLE 3 *Cutaneous uretero-ileostomy Post operative complications (25 patients).*

Operative death (peritonitis)	1
Intestinal obstruction	3
Stomal stenosis	2
Prolapse of ileostomy	2
Stomal bleeding	4
Minor ileostomy problems	5
Purulent bladder discharge	3

The followup period ranges from 1 to 6 years. The average followup time is 4 years. The patients were examined at 6-months intervals. The postoperative evaluation included, in addition to standard laboratory studies, excretory urography ileocystography cinefluorography of the urinary tract, serum electrolyte determinations and renal function tests. The patency of the stoma was tested and residual urine in the ileal bladder was mea sured. There was one fatality in the late postoperative period.

This was the case of an 18 year old girl with marked renal impairment, who for several years refused operation for fear of an esthetic handicap. When she finally accepted the operation she was totally incapacitated with febrile pyelonephritis. Her symptoms of acute infection subsided postoperatively but she died of progressive renal failure.

TABLE 4 *Cutaneous uretero-ileostomy Pre and postoperative pyelographic findings (23 patients)*

	Preop.	Postop.
Normal	14	19
Upper urinary tract:		
Slight dilatation (grade I)	8	10
Moderat dilatation (grade II)	7	11
Marked dilatation (grade III)	17	6

Representing 46 individual renal units.

Table 5 Cutaneous ureteroileostomy Pre and postoperative blood urea and electrolyte determination (23 patients).

	Preoperative values		Postoperative values	
	normal	pathologic	normal	pathologic
Blood Urea	20	3	22	1
CO2	1	5	22	1
Serum chloride	20	3	--	1
Serum potassium	3	0	23	0

In evaluation of the late results in the remaining 23 patients the criteria used were the postoperative incidence and degree of hydronephrosis pyelonephritis and biochemical imbalance The preoperative and postoperative status of the upper urinary tract as determined by excretory urography is shown in Table 4 The pyelograms are tabulated as individual renal units Slight hydronephrosis is classified as grade I moderate as grade II and marked as grade III The number of normal pyelograms is greater after operation than before The number of grade I pyelograms is slightly increased postoperatively The number of postoperative grade II pyelograms is also moderately increased On the other hand the number of grade III pyelograms is considerably less after operation than before Twenty-nine of the 46 renal units involved in the 23 uretero-ileostomies are either normal or show a minimal hydronephrosis postoperatively Seventeen still show a moderate or marked hydronephrosis. This last group includes 6 patients who before surgery had advanced hydronephrosis febrile pyelonephritis and impaired renal function Two others showed improvement of hydronephrosis in the early postoperative period but were noted to have recurrent hydronephrosis at later examination. One patient with minimal preoperative hydronephrosis showed postoperatively progression. All patients with normal pyelograms preoperatively had unchanged pyelogram postoperatively Regression of hydronephrosis was observed in about one half of the patients

Ileocystography was performed in ... patients With one exception all demonstrated ureteral reflux

None of the patients had clinical signs of pyelonephritis postoperatively Fifteen, however have persistent pyuria and positive urine cultures

Preoperative and postoperative blood urea and serum electrolyte determinations are shown in Table 5 Only one of the 3 patients who had preoperative blood urea elevation showed no improvement Of 5 patients with preoperative acidosis one still has a nonprogressive asymptomatic acidosis None of the children had lowered serum potassium levels or symptoms referable to depletion of total body potassium

Discussion

Because of the anal dysfunction present in the majority of children with spinal lesions ureterosigmoidostomy is not feasible Moreover the greater risk of hyperchloremic acidosis and pyelonephritis following ureterosigmoid diversion makes this method undesirable Since Bricker's [5] description of the cutaneous uretero-ileostomy this method has been used in pediatric urology with increasing frequency [9 10 14 15 17 19 20 21 22, 25] Large pediatric series with long-term followup have not been available

however For this reason clear-cut indications for the use of the method are not yet established. Therefore each case must be judged carefully by evaluating the general prognosis, family background and possible psychological disturbances. The most important consideration, however should be the salvage of the urinary tract before irreversible changes develop

Progressive hydronephrosis and recurrent urinary tract infection with deterioration of renal function call for prompt surgery Unless effective urinary drainage is instituted the long term prognosis is extremely poor Until surgery is resorted to every effort should be made to support and preserve renal function In the presence of residual urine triple micturition or bladder neck resection, in addition to long-term chemotherapy should be used. Twelve of our patients had already had operations on the bladder in an attempt to reduce residual urine Intractable urinary incontinence which interferes with a normal social life calls for operation when conservative measures have failed. In 3 of our patients belonging to this group penis clamps and urinals were tried preperatively without success.

The isolated ileal segment must function as a conduit and not as a reservoir Stagnation of urine increases the risk of reabsorption and predisposes to infection and stone formation. A prerequisite for optimal function is thus the use of the shortest possible ileal segment This is particularly important in children in whom there is continued growth of the bowel. In this series the length of the ileal segment used ranged from 10 cm to 1_ cm. The ileal bladder may be placed either intraperitoneally or extraperitoneally This last position may reduce the risk of peritonitis in the event of urinary leakage This complication, however seems to be rather uncommon and even when it occurs it may cease spontaneously [8 0 14] In the present series the ileal bladder was placed intraperitoneally in all cases The obvious advantage of having a short and unobstructed *ileal loop more than* compensates for this minimal risk involved. The ileostomy should be constructed with the utmost care to avoid postoperative stenosis. Even with properly constructed ileostomies the emptying of the ileal bladder is slow [4]. This establishes the importance of a well functioning ileostomy and stresses th fact that the margin of safety in patients with high stomal resistance and impaired renal function may be narrow

Special attention must be given to avoid the possibility of obstruction by angulation of the left ureter as it passes through the mesosigmoid. For this reason the left ureter should be passed under the root of the mesosigmoid rather than through it [8 14 24].

Calculus formation in the ileal bladder has been reported as a possible complication [* 6 0] In our series this complication has never occurred.

The incidence of postoperative hydronephrosis is low in all published reports [" 8 9 16 18 4] The best results were obtained in patients who had normal or slightly dilated upper urinary tracts preoperatively [14 _3]. The findings from this study concur with those reported in the literature Persistence of hydronephrosis or occurrence of hydronephrosis postoperatively is usually due to an imperfect anastomosis or ileostomy stenosis.

In some cases ext[illegible]ve ureteropelvic fibrosi[illegible] may be resp[illegible]ible. Since the progression of hydron[illegible]phrosi[illegible] may be silent frequent postoperative examinations of the urinary tract becomes mandatory. Two patient[illegible] who showed regres[illegible]ion of hydronephrosi[illegible] [illegible]x months after operation were subsequently found to have recurrent gros[illegible] hydronephrosis. One of these patients had an obstructed ileostomy but in the other no apparent cause of the dilatation wa[illegible] found. A third patient with minimal hydronephrosi[illegible] preoperatively has developed a moderate hydronephrosis 1 year following operation. In this case also no obviou[illegible] reason for this complication was found.

The incidence of pyelonephritis following uretero-ileostomy was reported [illegible] Bricker and associates [7] at [illegible]0 [illegible]. Increasing experience with the indication and technique of the method has resulted in reduced incidence of this complication (Cordonnier & Nicolai 1960 – 8.5 [illegible] Kerr *et al* 196[illegible] – 3 [illegible]). Thi[illegible] [illegible] a definite improvement over the [illegible]0 [illegible] incidence of infection reported in the use of ureterosigmoidostomy by the Mayo Clinic group [3]. Th[illegible] absence of postoperative febrile pyelonephritis in this series is perhaps the most impressive finding. An appreciable number of patients still have infected urine. The actual incidence of silent progressive pyelonephritis in this group can only be established by future observation.

In spite of the absorptive capacity of the ileum biochemical aberrations have been demonstrated to be less common than in ureterosigmoidostomy. This is partially explained by the short length of the segment used and its continuous emptying [15]. Acidosis a common complication of ureterosigmoid transplants is a rare complication of uretero-ileostomy. In 13 cases of uretero-ileostomy reported by Kerr *et al.* [13] there were 10 cases of acidosi[illegible] all limited to patient[illegible] with severe renal impairment. Of five patients in the present series with preoperative acidosis only one failed to improve postoperatively. The child still has gross hydronephrosis and severely impaired renal function. Hypokalemia following ureterosigmoidostomy is a well documented complication [1 2]. In our group there were no abnormal serum potassium values or symptoms referable to depletion of total body potassium. This is in accord with previously reported observations [11].

The disadvantages of uretero-ileostomy have been the higher incidence of operative complications and the need for an external appliance. Intestinal obstruction represents the most common complication in all publi[illegible]hed material. This fact emphasizes the need for special attention to the technique of isolation of the bowel segment and the intestinal anastomosis. Herniation of the small bowel around the ileal conduit is not uncommon [9 14]. This may be avoided by suturing the peritoneum to the segment as it enters the defect in the abdominal wall. Superficial stomal stenosis is usually due to irritation of the everted mucosa and squamous metaplasia of the epithelium. True mural stenosis involves the distal part of the ileal bladder and is due to inadequate excision of the abdominal wall. This complication may become a difficult problem requiring repeated revision.

Difficulty in maintaining a water tight seal between the stoma and the collecting device is a common experience. In our

series very few complications referable to the management of the ileostomy bag have been observed. Most patients adjusted themselves quite well to the care of the device and were able to wear the bag for as long as a week without changing it.

Psychologic reactions to the operation and to the wearing of an external appliance were observed in two of our patients. Careful preoperative psychologic preparation and patient reassurance can obviate much of this difficulty.

Another disadvantage of the method is the high incidence of ileo-ureteric reflux. This, however, occurs when the contrast medium at X ray examination is injected under pressure. Theoretically, in the absence of obstruction, there is no reason to believe that reflux occurs. Klinge & Bricker [15] have shown that the isolated ileal segments empty by periodic peristalsis initiated by hydrostatic pressure within the segment. With properly constructed segments it can be expected that emptying will be unimpeded and that there will be a minimal rise in intraluminal pressure.

Summary

Twenty five cases of cutaneous uretero-ileostomy in children aged from 2–18 years were reviewed with particular regard to postoperative complications, mortality and late functional results. One of the two deaths in this series was directly attributable to the operative procedure. The other occurred later as consequence of progressive renal damage. The surviving 23 patients showed a minimum of major complications. The greatest number of minor complications were related to stoma problems and the function of the collecting device. Average followup was 4 years. Twenty nine of the 46 renal units involved in the 23 uretero-ileostomies were either normal or showed a minimal hydronephrosis postoperatively. Seventeen still showed a moderate or marked hydronephrosis. None of the patients developed signs of clinical pyelonephritis postoperatively. Laboratory evidence of hyperchloremic acidosis was observed in one patient with advanced renal impairment. All patients were satisfied with this type of urinary diversion, which enabled them to maintain unimpeded social and educational activities. Although results appear promising final judgment should be reserved until long term followup studies are available.

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Department of Pediatric Surgery
Karolinska Sjukhuset
Stockholm 60
Sweden

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From The Paediatric Clinic University of Gothenburg Gothenburg, Sweden

Renal Acidification Defect in Infants with Mild Deficiency Rickets

by JAN WINBERG and TORE BERGSTRÖM

In the middle of the twenties BURGESS & OSMAN [6] and GYÖRGY KAPPES & KRUSE [11] demonstrated a lowered carbon dioxide combining power of the blood in patients with active deficiency rickets BURGESS *et al.*—discussing the origin of the acidosis—clearly defined the question when they stated that the low CO_2 combining power should be due either to an accumulation of organic acids in the blood or to a renal loss of alkali —the term at that time—or to both factors. The significance of this acidosis for the development of rickets was discussed with some eagerness, but with the general acceptance of rickets as a vitamin D deficiency disease these early observations lost their interest and studies mainly focused on calcium and phosphate metabolism, a field 40 years later still filled with controversies.

Renewed interest in metabolic changes of deficiency rickets—other than that of calcium and phosphate metabolism—have been rewarding. HARRISON & HARRISON in 1955 demonstrated characteristic alterations of citrat metabolism [13]. Experimental studies have lately suggested a disturbance of the Krebs cycle metabolism [22 23 25 28] Renal function studies have revealed different disturbances in proximal tubular function [5 1 17] But —to the best of our knowledge—the hypothesis of a disturbance of the renal regulation of acid base metabolism—set forth by Burgess *et al* forty years ago [6]—has not been tested with modern methods of renal function studies.

It was the object of our study to test different aspects of the hydrogen ion secretion capacity with aim of extending the knowledge of the diverse metabolic defects that seem to characterize deficiency rickets. The findings suggest that even in mild rickets, there is a defect distal tubular function, although in the infants investigated it had not caused a derangement of acid-base equilibrium.

Material

Thirteen infants aged 4–13 months with deficiency rickets. Vitamin D although in insufficient amounts, had been given to all patients but one who had received no prophylaxis at all. Eleven were admitted because of spasmophilia, two because of infections. The roentgenological skeletal changes were slight or minimal. The increase in serum alkaline phosphatase activity was rather moderat in most patients. Biochemical details are given in Table 1

Methods

Urinary pH was measured in a Beckman pH meter; blood pH and standard bicarbo-

TABLE I. *Some Serum and Urinary values in 13 rachitic infants*

Case	Age (mon.)	pH	pH	Stand. bicarb. mEq/L	Conc C p. mOsm/L	Ca	P	Alk Phosphatase units	Dose of Vitamin D given (I.U.)	Amino N mg per 100 mg creatinine
1 610225	4	5 4	7 44	40	—	9 4	2.7	64	90 000	111
E. F ♂		4.5				10	5.3	72		47
610529		6.1	7 40	29	—	7.0	2.8	79		—
M. L		5.4				9.8	5.9	39	→ 140,000	—
		4.9				11 0	6.6	—	+ 90 000	—
2 61 [illegible]	1	—	41	40	—	8.4	2.4	32	150 000	—
S. T		4 7				9	5 9	55		
4 61093	11	5 4	7 49	22	6 3	.2	4 9	35	400,000	62
M. F		5.0				9 4	5.5	—		—
		4 9							+ 30 000	21
5 810	8	5.8	7 53	24	904	8.	3.0	30	400 000	81
J. K ♂		4 6				10 4	5.2			
61 515	9	4 8	41	22	753	5.6	6.2	21	400,000	4
L. M ♂		4.				8.4	6 0			12
6101	13	5.3	43	21	860	9 8	2.6	4	300 000	34
I. S		4 6								31
8 61103	6	4 6	—	—	—	7 4	6.7	49		
L. S	—								—	—
9 621 1	6	5.1[b]	7 44	17	—	5.6	3	4	140 000	78
B M O ♀		4 4				10.6	5.5	46		83
10 620815	5	5.5	47	—	—	6.4	4.8	31	65 000	98
D J ♂		5 1				10 8	7.0	30		97
11 640904	6	5.4	41	22	—	6.2	4.5	37	—	35
P K ♂										—
12 640079	6	5.5[c]	35	20	—	5.0	6.6	37		—
R. B ♂									—	—
13 62051	11	5.8	40	3	—	6 8	4 4	23	180 000	6
I O ♂		4.3				10.0	5.9			41

First values in each patient gives the condition during first NH_4Cl load.
Second values in each patient gives the condition during second NH_4Cl load.
Normal values during first year of life below 70 units.
Lower values observed (Case 9–4.8; Case 11–4 8) but only following parenteral administration of aneurin and pantothenic acid.
Lower values were observed, but only immediately after intravenous calcium infusion (see Fig. 15).
[d] The physiological variation of urinary amino nitrogen excretion in infants has been given by Jagenburg [16].

nate according to Astrup *et al* [ ]; serum alkaline phosphatase activity with a modification of the BESSEY & BESSEY [7] method. Amino nitrogen in urine was determined by a colorimetric ninhydrin method [16]. Amino acids in the urine were separated by paper chromatography. Other blood and urine analyses were performed with conventional laboratory methods. The excretion of different urinary constituents has been expressed in relation to "true" creatinine excretion.

When the ability of the patients to lower urinary pH was examined the following technique was applied. Ammonium chloride was given in a dose of 5 g—about 90 mEq—per square meter body surface area per 24 hours. The load was given during 3–5 days as a rule. Individual doses were given four

[1] The authors express their thanks to Dr J. Oxvstad, Department of Medical Biochemistry, University of Gothenburg for these determinations.

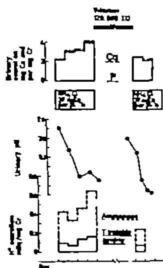


Fig. 1 Case 3; 10 months old girl with rickets, cf Table 1. Renal acidification capacity urinary hydrogen ion, calcium and phosphate excretion per mg creatinine during an NH_4Cl load, before and after treatment with vitamin D Day 1 means the first day of ammonium chloride load. Acidification capacity was less effective before vitamin D than after H excretion—certainly ammonium excretion—was rather high before Vitamin D in spite of high urinary pH. This was especially obvious the first day of the pretreatment period.

times daily usually at 6 a.m., t noon, at 6 p.m. and t midnight pH of urine was estimated in samples if possible collected before the morning meal between 8 and 9 o clock. In some instances pH of the 4 hour urine volume was also determined. Renal concentration capacity was determined fter injection f pitressin tannate in oil[3] [29].

Results

The ability of 13 infants with deficiency rickets to lower urinary pH during an NH_4Cl load is shown in Table 1

Before treatment with vitamin D the minimum pH observed was above 5 (range 5 1–6.1) in 11 patients, and below 5 (4.8–4 6) in two cases, nr 6 and nr 8. After about 10 days treatment with vitamin D the acidification capacity was reinvestigated in eleven patients. Now pH values below 5 (range 4.3–4.9) was observed in ten Only one showed a value above 5 (Case 10) In this case only 65,000 I U of

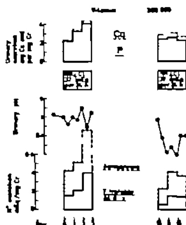


Fig 2. Case 5; 8 months old boy with rickets, cf Table 1 Registration as in Fig. 1 Defect acidification capacity before itamin D Note also high excretion of H before treatment.

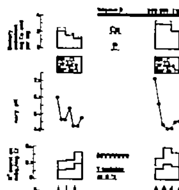


Fig. 3. Case 6; 9 months old boy with rickets, cf Table 1 Registration as in Fig. 1 Acidification capacity about equal before and after vitamin D But the drop of pH more rapid after treatment. The rickets was in this case very slight and the excretion of amino acids was within normal limits.

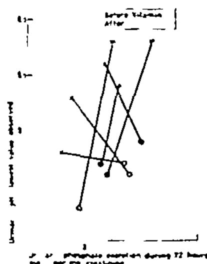


Fig. 4 Cases 3–8 and 13. Urinary pH compared with phosphate excretion in mg P per mg creatinine during hours NH Cl load before and after vitamin D pH lowest values observed during the loads. There seems to be no correlation between the amount of phosphate excreted and the ability to produce a urine with low pH
1) Case 3 Excretion measured during 48 hours only

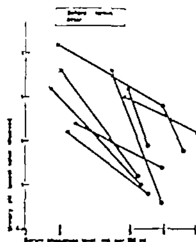


Fig. 6. Cases 1 13. Urinary pH compared with serum phosphate level. pH: as in Fig. 4. Serum values determined at the time of the acid loads. No patient with a phosphate level below 5 mg per 100 ml lowered pH below 5. Three patients with normal serum phosphate levels (5.8, 6.6, 7.4) lowered pH only to level between 5.4 and 5.1

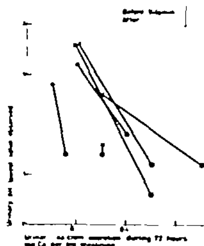


Fig. 5. Cases 3–8 and 13. Urinary pH compared with calcium excretion in mg Ca per mg creatinine during 72 hours NH$_4$Cl load before and after vitamin D pH as in Fig 4 In the individual cases there seems to be positive correlation between calcium excretion and ability to lower pH of urine However at a given calcium excretion there may be found cases both with and without ability to lower pH below 5.
1) Case 3 Calcium excretion determined only during the second 24 hours of the acid load

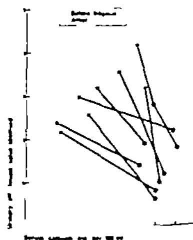


Fig 7 Cases 1 13. Urinary pH compared with serum calcium level. pH as in Fig 4 Serum values as in Fig 10. The findings are complex and not easily interpreted. In most cases normalization of a low serum calcium level was accompanied by an improvement of acidification capacity But three patients still did not lower pH below 5.4 5.3 resp. 5.1 in spite of normal serum calcium level (9.8; 9.8 10.8). Two patients lowered the pH of urine efficiently in spite of rather low serum calcium levels (8.8 and 7.4 mg per 100 ml resp.).

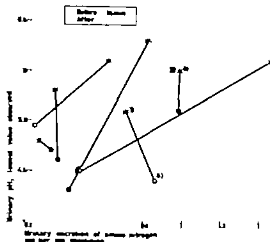


Fig. 8. Urinary pH compared with amino acid excretion expressed as amino nitrogen per mg creatinine pH₁ as in Fig 4 Amino acid excretion usually determined during 24 hour period at the time of the acidification tests. Although most patients with high (>0.4 mg amino N per mg creatinine) excretion were incapable of lowering the pH below 5, there seems t be no direct correlation between amino nitrogen excretion and ability t lower pH of the urine. The figures refer to the number of the cases.

) Determination of pH after parenteral administration of aneurin and panthotenic acid.

vitamin D had been given when the second acidification test was performed. In the other patients the dose varied between 120 000 and 200 000

Details of the cidification tests in three patients are given in Fig 1–3. It is seen among other things that although there were no noticeable changes in diet H⁺ excretion in the two patients with an acidification defect Fig 1 and 2, seemed to be lower after treatment than before It is noticeable that the reduction of ammonium excretion occurred in spite of lowered minimum pH (cf also Fig 9)

Phosphate excretion increased or decreased after treatment with vitamin D while calcium excretion was unchanged or increased, Fig 4 and 5 Amino acid excretion decreased markedly or was virtually unchanged (Table 1 Fig 8)

Urinary output of sodium, potassium and chloride before and after vitamin D is

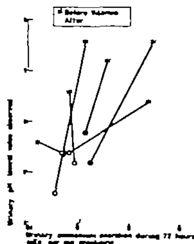


Fig 9. Cases 2–8; 12. Urinary pH compared with ammonium excretion in mEq per mg creatinine during 72 hours NH_4Cl load before and after vitamin D pH₁ as in Fig. 4. Although there was in most cases marked fall of pH after treatment with itamin D no corresponding increase in ammonium excretion was observed. Rather the inverse When the pH of the 24 hour specimens were plotted against the 24 hour ammonium content of the urine the same impression remained.

1) Cases 2 and 7; Ammonium excretion determined during 24 resp. 48 hours.

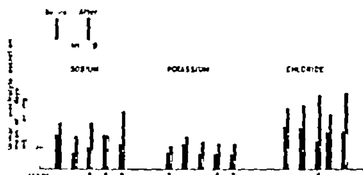


Fig 10. Urinary excretion of sodium, potassium and chloride in mEq per mg creatinine during 7 hours NH Cl load before and after vitamin D (Case 3 48 hours oral).
In all cases except nr 6, who also had normal acidification capacity and a low amino acid excretion, there was an increase in electrolyte excretion after vitamin D. Intake of electrolytes was about the same before and after treatment

compared in Fig 10 As can be seen in all cases but one pretreatment excretion was less than posttreatment excretion. Increased electrolyte intake could not explain the observed changes Case 6 was the only patient in whom pre and post treatment excretion were equal This was one of the two infants who were able to lower pH below 5 before treatment

Lastly the renal concentration capacity after pitressin administration was studied in four patients (Cases 4–7 Table 1) and found to be within normal range before treatment [20].

When the ability to lower pH was correlated to the different factors studied the following relations were found. The only two patients with an initially good capacity of acidification had rather low serum alkaline phosphatase activity Otherwise no parallelism was found between the severity of the rachitic process, as measured by serum alkaline phosphatase activity and the minimum pH observed (Table 1). Nor was there any definite correlation between acidification capacity and urinary phosphate and urinary amino nitrogen excretion, Fig 4 and 8

On the other hand the increased acidification capacity in each case was found to run parallel to an increase in serum calcium and serum phosphate levels (Fig and 6) and an increase in urinary calcium excretion (Fig 5) The following facts however merit observation. *A* Three patients with normal serum phosphate levels 5.0- 6.0 7.0 mg per 100 ml lowered pH only to between 5.1 and 5.4 (Table 1 Cases 9 10 and 12) *B* Three patients with normal serum calcium levels 9.8, 9.8 10.8 mg per 100 ml lowered pH only to between 5.1 and 5.4 (Table 1 Cases 7 and 10) *C* Two patients with low serum calcium levels—5.8 and 4 mg per 100 ml —lowered pH to 4.8 and 4.6 (Table 1 Cases 6 and 8) *D* At a given amount of calcium excretion there may be found patients *with* and patients *without* ability to lower urinary pH below 5 (Fig 5)

These observations suggest that the improvement of the acidification capacity on one hand and the normalization of phos-

phate and calcium levels in serum on the other may be different expressions of an improvement in the rachitic process, but otherwise not causally related. The significance of the urinary calcium excretion will be dealt with later.

Some preliminary studies over the effect of I.V. calcium on the urinary acidification deficiency have been performed. In Case 12 (Fig. 11) treatment was started with an intravenous calcium infusion and urinary pH was measured. Pretreatment values were alas not obtained, but it is obvious that pH after the initial calcium infusion was lower (pH 4.9) than during a consequent NH_4Cl load (pH 5.3). Two further calcium infusions were followed by a rapid decrease in pH and a subsequent increase. In this patient acidification capacity was best correlated to urinary calcium excretion.

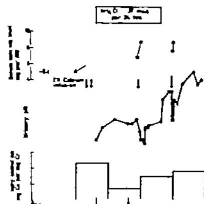


Fig. 11. Case 12, 6 months old boy with rickets, cf. Table 1. Urinary pH, serum calcium level and urinary calcium excretion after intravenous calcium *infusions and during an NH_4Cl load. In* each of the first three infusions 4 ml of a 10 solution of calcium gluconat was given, in the fourth infusion 6 ml of the same solution. The rapid fall of urinary pH after the two last infusions suggest causal association to the calcium administration. A similar response t the first infusions may only be presumed since no pretreatment values of the urinary pH were obtained. The ability to lower urinary pH may be better related to urinary calcium excretion than to serum calcium level.

Discussion

In forming urine of maximal acidity pH 4.4 from blood plasma, pH 7.4 hydrogen ions are secreted against a concentration gradient of 1:1000. Urinary pH of 4.5 and 5.4 correspond to concentration gradients of 1:800 and 1:100 respectively. The moderate difference in terms of pH between pre- and posttreatment acidification capacity observed in the rachitic infants of this study thus means a marked deficiency in terms of ability to establish hydrogen ion concentration gradient. When phosphate is the main urinary buffer—with a maximal buffering effect at a pH between 6 and 7—tubular defects of the order found in this study do not influence the acid base equilibrium of the body fluids.

Deficiency rickets may be associated with multiple changes of renal tubular function such as decreased reabsorption of phosphate [12], aminoacids [17] and glucose [5]. To this is now added an insufficiency of tubular acidification capacity. Thus the whole spectrum of the de Toni Debré-Fanconi syndrome may be found in deficiency rickets, although the changes seem to be much more marked in the classic form of the former condition. There are similarities also to another tubular disorder. An acidification defect associated with an unexpectedly high excretion of ammonium ions is namely found in primary tubular acidosis [*6].

Deficiency rickets thus has some features in common with two tubular dis-

orders both of which are usually associated with a skeletal rickets characterized by a conspicuous resistance to vitamin D.

The pathogenesis of the earlier known tubular disorders in deficiency rickets is still under discussion. A direct consequence of lack of vitamin D in tubular cells cannot be excluded since labeled vitamin D—or metabolites thereof—have been shown to accumulate at the site of reabsorption of phosphate, aminoacids and glucose in the proximal tubules [9] as well as in most other structures where vitamin D has been shown to exert a physiologic effect [18]. But in the distal part of the tubules, where hydrogen ion secretion mainly takes place [24], labeled vitamin D does not accumulate [19]. Thus other explanations than an in loco deficiency of vitamin D should be considered especially in a discussion of the pathogenesis of the defect acidification capacity.

The most probable explanation of this defect would seem to be a buffering effect of a massive phosphaturia. The increase in phosphaturia seen in three out of six patients in spite of improved acidification capacity renders this explanation improbable.

Intravenous infusions of calcium seemed to cause a fall of urinary pH in one patient. This effect of calcium has recently been observed in healthy adults [1, 27]. In our patients the acidification capacity was, however, not related to the serum level of total calcium. But the concentrations of the different calcium fractions in serum [4] may have been more important. A certain correlation to urinary calcium excretion especially in the patient infused with calcium may suggest that it is the saturation of the renal tubular system with calcium which influences the ability to establish a high H+ concentration gradient. The role of magnesium also deserves consideration [3].

The origin of the acidosis sometimes found in rickets is still obscure. This investigation suggests that it may have a renal origin although the defects found in the patients of this study are too small to influence acid base equilibrium.

Another explanation would be an accumulation of acid metabolites. The high excretion of hydrogen ions in some of our patients—already described in active rickets in 1911 by HODGSON [15] and 1922 by FREUDENBERG & GYÖRGY [10]—could speak in favour of this. The reports of a disturbance of the Krebs cycle metabolism in experimental rickets [22, 23, 25, 29] possibly deserves consideration in this context. Such a fundamental metabolic disorder—if it exists in human rickets—could of course explain also the renal tubular defects in several ways, i.e. by a toxic effect of an accumulated metabolite [21].

The increase in sodium excretion is so constant that it has to be considered as significant although the mechanism is uncertain. One explanation may be that sodium located on the surface of bone crystals [20] during healing are exchanged with calcium ions and excreted in the urine. Increased excretion of potassium and chloride may have a similar explanation. A pure renal origin of the increased electrolyte excretion cannot, however, be excluded.

Summary

1. A deficient renal acidification capacity was demonstrated in infants with mild

deficiency rickets. In terms of ability to establish a high hydrogen ion concentration gradient between tubular cells and tubular lumen the defect was considerable. Hydrogen ion excretion was not impaired.

2. The ammonium levels in the urine proved in some untreated patients to be higher than that of the urines of equivalent pH of the treated subjects.

3. Intravenous calcium infusion seemed to increase the renal acidification capacity.

4. The pathogenesis of the defect is discussed with regard to other metabolic changes observed and to the physiology of vitamin D.

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Summary

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From the National Bacteriological Laboratory the Department of Virus Research, Karolinska Institutet School of Medicine Stockholm, and the Children s Health Center Karlskoga, Sweden

Smallpox Vaccination Studies with Serial Dilutions of Vaccine

III Comparison of Take Rates in Two Age Groups of Infants (Less than 10 Weeks and 5–12 Months Old Respectively)

by J ÅKE ESPMARK and ERIK RABO

Introduction

Results of smallpox vaccination in very young infants have been reported by several authors in the past [3 4 11 14 15 17]. These reports have been mostly in agreement with respect to two important features which distinguish the vaccination response in the youngest infants from that found in older children. In very young infants the vaccination often fails to take nd, in case of success, the local and general reaction usually runs a mild course Those characteristics, which seem t indicate an increased resistance both to the establishment of infection and to its further development have been ascribed to the presence of passively transferred, circulating maternal antibody [1 4 13]. A similar increase in resistance to vaccinia virus has been achieved also in animal experiments with controlled administration of antibody by injection [2, 16] and in human vaccinations combined with large doses of immune gamma globulin [6 1].

In a study of the proportions of takes in human adults vaccinated with serial dilutions of vaccine Espmark [5] found that the relative susceptibility of a population, homogeneous with respect to vaccination history could be characterized by the position of the sigmoid response curve in relation to the dose scale This study was thought to offer some basic information by which to define certain potency requirements.

The aim of the present work was to apply the same dose response method with a similar intention, to study the susceptibility of a population of young infants (less than 10 weeks of age) Comparison was made with a group of older children (aged 5–1 months) A short summary of the results of this study has been reported previously [7].

Material and Methods

Children. The material was collected during a one-year period from infants routinely attending a children health center According t age at the time of vaccination, the infants to be included in the study were classified in the following age groups:

Young infants less than 10 weeks old (age range 25–70 days).

Older infants 5–12 months old.

All vaccinations were primary and no repeated attempts in case of failure to take were included in the study.

Vaccine. The same glycerinated calf lymph vaccine (CJ 831), was used throughout the trial, where not otherwise stated in the text. Serial half-log dilutions (1:3.16, 1:10, 1:31.6, ... 1:1000) were prepared using saline with 0.35% agar as a diluent. In addition the dilution 1:6, as used in public vaccination, was included in the study. Dilution series were prepared on three different occasions during the test period. The dilutions were filled in glass tubes or plastic ampoules and stored at −35 C until mailed to the vaccination station in ice-chilled thermos bottles. Vaccine shipments were arranged about twice each month. Use was made of good refrigeration and deep-freezing facilities at the vaccination station.

The titer of the undiluted vaccine was $10^{6.8}$ $TCID_{50}$ per ml in monkey kidney tubes.

Vaccination and reading of results. All vaccinations and readings were performed by the same person (F. R.) except in the 46 children represented by the upper part of Table 1 (performed by Å. L.). Inoculation by multiple pressure was made on two sites about 5 cm apart in the left scapular region. As a rule two different vaccine dilutions were applied in each child. At reading after one month the result (take or failure) was recorded for each vaccination site separately.

Statistical treatment. Take frequencies (= quantal responses) obtained with the various vaccine dilutions were analysed by methods described by D. J. Finney in his book *Probit Analysis* [10].

Results

From a previous study [6] it was learnt that in revaccinations the effect of a weak vaccine was not markedly influenced by a strong vaccine applied simultaneously at a different site in the same individual.

TABLE 1. *Tests of possible interaction between two vaccine dilutions of different potency applied in the same individual in primary vaccination*

Size of subgroups (No. of indiv.)	Vaccine dilutions inocul. into each child	Frequency of positive reactions	Per cent pos.
46 children aged 4–10 years (Vaccine E 93)			
[illegible]	undil.	[illegible]/20	
	1:100	18/20	[illegible]
[illegible]	1:100	23/26	[illegible]
	1:100	23/[illegible]	
23 children aged 3–10 weeks (Vaccine CJ [illegible])			
11	undil.	11/11	
	1:100	6/11	[illegible]
11	1:100	4/11	41
	1:100	6/11	

Although it seemed likely that the same would hold also for primary vaccinations a couple of small control tests were made to corroborate this assumption. In each test a group of individuals was divided into two subgroups, the one being given a weak vaccine plus a strong vaccine, the other the weak vaccine on both sites. The proportions of takes with the weak vaccine in the two subgroups were compared. The results as seen in Table 1 did not suggest any interaction of the kind indicated above.

Consequently two different vaccine dilutions were thereafter applied to each individual and the two responses were considered to be independent of each other.

The success rates obtained in the two age groups are shown in Table 2. As seen, there were about twice as many infants in the young group (10 weeks old) as in the older (5–12 months old). The number of dilutions applied in the two groups were

TABLE ... *Smallpox vaccination take rates obtained with serial dilutions of vaccine in two age groups of infants*

Frequencies refer to vaccination sites. Titer in monkey kidney tubes of undiluted vaccine: $10^{8.8}$ TCID$_{50}$ per ml.

Dilution of vaccine	Age less than 10 weeks a/n	Per cent positive	Age 5–12 months a/n	Per cent positive
Undiluted	62/67	94	—	—
1:3.16	53 64	83	—	—
1:6	59/67	88	—	—
1 10	51/66	77	37/37	100
1:31.6	51/79	65	46/50	92
1 100	22/90	24	49/67	72
1:316	2/49	4	19/57	33
1 1000	—	—	8/29	21

a/n = Number of takes through number of inoculated sites.

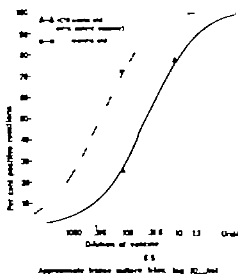


Fig. 1. Take percentages in smallpox vaccination of young infants (<10 weeks old) and older infants (5–12 months old) using serial dilutions of vaccine

7 and 5 respectively The regular decrease of the proportions of takes with diminishing vaccine potency is apparent for both age groups. When success rates with vaccine dilutions common to both groups (i.e. 1 10–1 316) are compared, a marked difference is observed, the corresponding percentage range in the younger group being 77–4 %, in the older group 100–33 %.

The same data are illustrated in diagrammatic form in Fig 1 where take percentages are plotted against a logarithmic dilution scale and the best fitting normal sigmoid curves (obtained by probit analysis) have been drawn to represent each point sequence No mathematical test of normality of the point series was made but as may be seen in Fig 1 the deviations of points from the normal curves do not exhibit any definite trend which would suggest a clearly better fit to any definable alternative distribution.

After these vaccination series were furnished an extra potent vaccine for revaccinations" (titer $10^{9.8}$ TCID$_{50}$/ml) was used in 100 consecutive vaccinations in infants under 10 weeks of age. The number of takes was 198 – 99 %. This point has been entered in the diagram of Fig 1 but was not included in the following probit analysis.

Details of the probit analysis will not be given, but attention will be paid to some statistics characterizing such properties of the points series as homogeneity and parallelism, steepness of the curves, estimated 50 % doses, and dose distance between the curves.

The probit analysis provides the sum of the squared deviations of the response variable from the curve This residual sum of squares may be used as a chi-square in testing goodness of fit of the points to the postulated curves and the parallelism of the curves. For details of calculations

TABLE 3. *Analysis of chi-squares (sums of squared probit deviation from fitted regression lines) for testing heterogeneity and departure from parallelism of dose-response data obtained in smallpox vaccinations of young infants (< 10 weeks) and older infants (> 1[illegible] months)*

Source of heterogeneity	Degrees of freedom	Sum of residual squares	Mean square
Parallelism of regressions	1	1.77[illegible]	1.772
Residual heterogeneity	8	23.44	[illegible]
Younger infants	5	1[illegible].8[illegible]0	3.361
Older infants	3	5.622	1.874
Total	9	[illegible]	[illegible]

reference may be made to Finney's book "Probit analysis" [10]. The chi-square analysis is summarized in Table 3. Since the normal expectance of the mean squares in this test is unity, the figures in the last column of Table 3 indicate some heterogeneity of the data, shown by partitioning to be located mainly in the group of young infants (cf. Fig. 1). The mean square for parallelism is less than the mean square of residual heterogeneity, indicating that no doubt need be raised concerning the legitimacy of fitting parallel curves. The mean square of heterogeneity (= [illegible]03) is taken as the heterogeneity factor, by which all variances are multiplied to compensate for under-estimation of variation. The slopes of the probit lines in the present study, −1.40 and −1.22 (with the weighted average −1.20), indicate a flatter course than was previously found in similar experiments in adults (average −1.60). This flatter course implies that variation is relatively large. It may be suspected that this variation originates not only from differences in susceptibility among the infants, but also partly from departure of the actual potency of the vaccine dilutions from the strength expected by their degree of dilution. Such dose errors might have been produced through the preparation of new dilution series, which was done twice during the experiment, or through possible deterioration during transport or storage, etc. The equal deviation in both age groups of the take rate obtained with the dilution 1:316 may e.g. be suspected to be a result of a systematic dose error (cf. Fig. 1).

Although not statistically significant, visual inspection of the course of the point series suggests that the variation is larger among the younger infants. Such would in fact be expected on account of varying immunity level in the mothers and a steadily changing susceptibility due to decrease in the level of maternal antibody at this age.

The relative susceptibility of the two age groups is measured by the dose distance between the response curves. Fifty per cent takes is given by an estimated vaccine dilution of 1:35 in the younger group and 1:[illegible]63 in the older, corresponding to tissue culture titers (monkey kidney) of about $10^{[illegible]}$ and $10^{[illegible]}$ $TCID_{50}$ per ml respectively. The difference is $10^{[illegible]}$ = 7.6 times with a standard error of $10^{[illegible]}$, giving a 95% confidence interval of 3.8–15.4 times.

Discussion

The advantages and drawbacks of smallpox vaccination in early infancy have been discussed by many authors.

As credits have been considered the milder local and general reactions. For

thermore the growing schedule of other immunizations starting at three months of age have made it desirable to have small pox vaccination completed by that time if it can be safely and effectively done

Besides some possible disadvantages of an immunological character the most advocated objection in practice has been the difficulty of attaining a satisfactory take rate in very young infants. This last question alone has been treated in the present study

Application of serial dilutions of vaccine in two infant age groups (below 10 weeks and 5–12 months) resulted in take frequencies which could be fitted to normal sigmoid curves with pproximately paral lel course when plotted against a logarith mic dose scale. From the dose distance between the curves it wa estimated that the younger infants needed a vaccine potency 7 to 8 times higher than the older

The vaccine potency corresponding to estimated 50 % takes was $10^{4.5}$ TCID$_{50}$ per ml (titer in monkey kidney tissue cul tures) for the younger infants, and about 10^{4} for the older and the corresponding titers expected to give 95 % takes were 10 [illegible] and about $10^{7.9}$ respectively

Of practical importance is the finding that a high take rate could be obtained also in the young infants provided the vaccine potency was high. An extra potent v ccine" (titer $10^{8.2}$ TCID$_{50}$ per ml) used later to vaccinate 200 infants of this age group gave 99 % t kes.

The dose-response relationships found in this work were similar to those obtained in a previous study on adult human popula tions using the same methods [5]. The present curves re flatter however indicating more variability especially among the younger infants. Some of this increased variation may be due to failure to keep the dose sufficiently stable as noted above In the younger group of infants wider variation is not unexpected, since within the range of ages of that group (usually 4–10 weeks) susceptibility is successively changing due to gradual decrease of maternal antibody A further source of susceptibility variation in young infants is the vaccination history of the mothers. Doorschodt [4] demonstrated that children of unvaccinated mothers did not exhibit any of the increased resistance to vaccina tion in the first few months of life, as otherwise observed if the mothers had been vaccinated. In the present study detailed vaccination histories of mothers were unfortunately not vallable, but it was estimated that somewhat more than 95 % had been vaccinated as a rule more than 15 years earlier

In a separate study on antibody formation in young infants [8] it was found that 23 out of *6 children vaccinated before the age of 35 days had appreciable titers of neutralizing antibody prior to vaccination. The antibody level 1 year after vaccination was only lightly lowe in this group than in a control group of 9–12-month-old children. These findings will be more thoroughly reported and discussed in another paper [9]. It seems very probable that the most important goal of routine vaccination, i.e. basic immunization, is easily chieved also in infants with maternal antibody

The practical implication of the find ings of this study is, that provided other disadvantages of vaccination of young infants can be excluded, early vaccination may be recommended if a lower take rate

TABLE 3. *Analysis of chi-squares (sums of squared probit deviations from fitted regression lines) for testing heterogeneity and departure from parallelism of dose response data obtained in smallpox vaccinations of young infants (<10 weeks) and older infants (3–7 months)*

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Total	9	25.21	2.80*

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From the Department of Pediatrics (Head: Jørgen Flamand Christensen) Odense County and City Hospital, Odense, Denmark

Metabolic Acidosis in Infantile Gastroenteritis

Physiologic and Therapeutic Aspects

by POUL KILDEBERG

The clinical history of acidosis in infantile gastroenteritis has its origin in early discussions of the metabolic characteristics of the so-called "Säuglings-Toxikose" undertaken by Keller & Czerny [6], Pfaundler [44], Ylppö [58], and others. However Howland & Marriott [24 25] are usually given credit as the first to have established, explicitly the concept of acidosis in infantile diarrhea The works of Howland & Marriott were followed by others [14, 17 18 20 22 49] reflecting the gradual improvement of clinical laboratory methods and the resulting growth in the knowledge of physiologic acid base homeostasis.

In this country Friderichsen [12] and later Kirk [35] have published extensive studies on this subject and further references to the early history of acidosis in infantile diarrhea are given by these authors.

During recent years few studies have appeared dealing, specifically with acid base balance in gastroenteritis but valuable information may be gathered from the numerous papers concerning parenteral fluid regimens [3 8 10 53 55] which followed the recognition of potassium deficiency by Govan & Darrow [13], and of hypertonic dehydration, by Rapoport [40], as important constituents of the metabolic derangement presented by many infants with severe gastroenteritis.

It is the purpose of this paper to provide further information concerning the nature frequency and treatment of the metabolic acidosis of infantile diarrhea, based upon a study of 70 cases of gastroenteritis treated at this clinic during the past three years.

Case Material

Two hundred and eighty four determinations of the acid base status were made in 70 consecutive patients (24 girls and 46 boys) with acute gastroenteritis, admitted to the Pediatric Department, Odense County and City Hospital, since May 1961 The age distribution and the results of the fecal cultures are shown in Table 1

Serum sodium values were obtained on admission from 42 patients. Three of these had values below 130 mEq/l, 27 presented with normotonic dehydration (serum Na 130-150 mEq/l), while initial values above 150 mEq/l were observed in 12 patients. Eleven infants with Coli enteritis presented with a normal serum K concentration.

There were no deaths.

Methods

Serial determinations of the acid base sta t s of capillary whole blood were made by means of the Astrup micro equipment (1 5). Duplicate equilibration were performed at both high (pprox 60 mm Hg) and low (approx 30 mm Hg) CO tensions, and actual pH values were recorded as means of two or more readings

Urinary net acid excretion (NAE) was measured by the method described by Jorgensen [*6], and urinary titrat ble acid (TA) was determined by titration to en l point pH 40 at pCO ~0 mm Hg [33].

Concentrations of N h and Cl in urine and plasma were supplied as routine measu rement by the Central Laboratory of this hospital. Further details of the analytical procedures are gi en elsewhere [30, 33].

In all cases of severe met bolic acidosis intra enous infusion of a 10 mEq/l solution of sodium bicarbonate was initiated soon after admission. With moderate degrees of acidosis, bicarbonat was likewise administered intravenously or in the absence of vomiting orally Initial doses were calculated according to the formula of Mellemgaard & Astrup [32 37] and infused in the course of 3–1 hours Following re-evaluation of the acid base statu treatment was continued if necessary but complet restoration of neutral ty in this way was not always at tempted. In hypernatremic infants, sodium bicarbonat was infused more cautiously usually in combination with isotonic glucose in water Administration f potassium was started when a sat sfactory flow of urine had been achieved but rather low doses were gi en during the first day cf p 164

Results

Fig 1 shows the acid base status a obtained on admission from each of the 0 infants and children of the present se ries. Eight patients (11 4) presented with severe metabolic acidosis (base excess (BE) < −18 0 mEq/l) 10 patients (22.0)

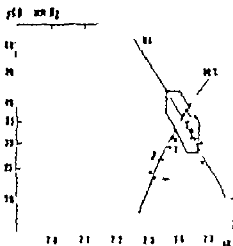


Fig. 1 A id base st tus on admission in 4 infants and young hildren with acute gastroenteritis. The hexagon indicates the normal range ($\bar{X} \pm s$) as computed from measurements mad in 64 normal infant peri 1 weeks Mean base excess (x), $\bar{X}$ = 1.3 mEq/l, standard deviation, 1.83 standard error of the mean, s_m 0 1932. Mean pCO_2, $\bar{X}$ 33.8 mm Hg 3.02, 0.422. Mean pH $\bar{X}$ 7 411 0.0—, 0.0031. The 50 compensation percentile shows the pCO_2 alue at which any non respiratory pH change is reduced by one half. NA Normal CO_2 bsorption line (Hb 13 g/100 ml).

were moderately acidotic (−19.0<BE < −10 0 mEq/l), while in eighteen (25.) only mild acidosis (−10 0<BE< −4 4 mEq/l) was demonstrated. In contrast with the findings in pyloric stenosis [22, 32], *6 patients (37 1) presented with a normal acid base status. In two cases slight metabolic alkalosis wa noted on admission possibly because of vomiting

As might be expected, the degree of acidosis showed no correlation to the duration of diarrhea, presumably because severe diarrhea usually causes more prompt admission. In infants with frequent aters stools, the degree of acidosis usually corresponded well with the clinical state of the patient but obviously many factors are involved, e g serum osmolarity po-



Fig. 2. Corresponding serum N and base excess (BE) values on admission in 42 infants and young children with acut gastroenteritis.

tassium deficiency number of vomitings, nd type and virulence of infection.

It is seen, in Fig 1 that in the majority of cases the acidotic values are grouped along the 50% compensation percentile described previously [31] but rather large deviations are apparent in several instances, especially in case of severe acidosis. However Fig 1 includes only values on admission, when general debility dehydration hypernatremia, prematurity and pulmonary and cerebral complications may easily obscure the respiratory effects of a decreased pH

Serum Na was determined on admission in 4 inst nces. Judged by this value serum osmolarity showed no pronounced correlation to the degree of acidosis (Fig), cf. discussion p 163

Fig 3–5 describe individual courses of the acid base parameters in three infants with gastroenteritis presenting with normo- hyper and hypotonic dehydration, respectively Fig 6 concerns a 3-month-old male infant in whom correction of the

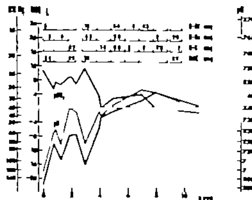


Fig. 3. Course of acid base parameters and urinary electrolyte excretion in 3-week-old male infant with Coli *6 enteritis and normonatremic dehydration. (NAE = Urinary net acid excretion.)

acidosis was achieved by intravenous infusion of THAM [40].

Fig. 3 gives acid base data of a 3-week old male infant with Coli *6 enteritis weighing on admission 3380 g He presented with a story of diarrhea and vomiting lasting 12 days and was found to be somewhat dehydrated with greyish, clammy skin and poor turgor but he was not shocked. The venous blood was

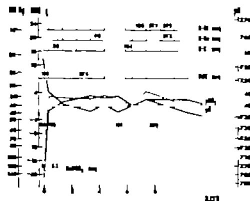


Fig. 4 Course of acid-base parameters and urinary electrolyt excretion in 6 month-old male infant with culture-negativ gastroenteritis and hypernatremic dehydration (NAE Urinary net acid excretion.)

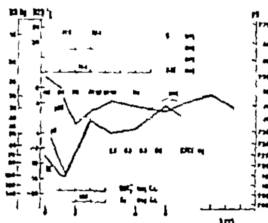


Fig 5 Course of acid base parameters and relevant electrolyt data of a 3-month-old female infant w th Coli *4 enteritis and hyponatremic dehydration. (NAE = Net acid excretion.)

concentrated and viscou (hemoglobin conc °1 g/100 ml hematocrit 63 vol.) and too little serum was prepared to per mit of further laboratory investigation.

Twenty hours after admission normal values of serum Na (13_ mEq/l) serum Cl (100 mEq/l) serum urea (30 mg/100 ml) and total proteins in serum (4 " g/100 ml) and a low serum K (*.5 mEq/l) were obtained. Serum Na and serum Cl concen trations remained normal serum K ri ing to 4 1 mEq/l on the third day

During the first day the patient passed only three small and not very liquid stool but during the following two days severe diarrhea acidosis, and clinical signs of dehydration recurred. Resumption of intravenous therapy (*vide infra*) was followed by permanent recovery After three weeks the infant was discharged weighing 4.3 kg

Treatment Fl id, electrolytes glucose ami no-acids, Arobon[1] tetracycline and neomycine

[1] The 5 solution used contains bout 11 mEq of potassium per liter

1st °1-hour period Oral fluids: Arobon (5). Intravenous fluids: Glucose (5.5), sodium bicarbonat (167 mEq/l).

Na. *3 4 mEq
K: _2 mEq
HCO_3: *8.4 mEq
Total fluid 800 ml.

nd 4-hour period Oral fluids Arobon (5), sod um bicarbonate (167 mEq/l) potassium chloride (51 mEq/l in 5.5 glucose).

Na. 20 0 mEq
K 15_ mEq
HCO_3: *0.0 mEq
Tot 1 fluids: 620 ml.

3rd 24-hour period Oral fluids. Arobon (3), potassi um lactate (51 mEq/l) in sodium lactat (100 mEq/l).

Na: 16.0 mEq
K 14 8 mEq
Lact te *4.2 mEq
Total fluids: 760 ml

4th 4-hour period Intravenous fluids: Glucose (5.5) Aminofusin[2] (3 % with electrolytes) sodium bicarbonate (167 mEq/l), potassium chloride (51 mEq/l in 5.5 glucose)

Na 25 0 mEq
K: 14 4 mEq
HCO_3 : 23 4 mEq
Total fluids: 1375 ml

5th and 6th day Grad al change from glucose (5.5) and Aminofusin intra enously and orally to Eledon[3] (5) orally

In this infant the re-establishment of a normal H balance involved three distinct metabolic phases: (1) An initial period of partial rehydration and continued losses of intestinal fluids (1st–3rd day) (2) A period of more efficient reparative therapy and increasing participation of the infant's own homeostatic functions (4th and 5th day) and (3) a postacidotic phase of mild metabolic alkalosis.

It is seen, from Fig 3 and the accompanying Table 2 that during the first days after admission the NAE values were un-

TABLE 1 *Distribution of 70 cases of acute gastroenteritis according to age and etiology*

Age	Etiologic agent: Coli 26	S. typhimurium	Coxsackie B5	Neg. culture	Total
< 2 weeks			1	2	3
2–8 weeks	11			6	17
2–6 months	10	1		19	30
6–12 months	2			10	12
1 2 years				4	4
2 3 years		1		2	3
3–5 years				1	1
Total	23	2	1	44	70

expectedly low in view of the BE (and pH) level being lowest in this period During this initial phase of partial rehydration the daily output of urinary net acid roughly covered the endogenous production of non volatile acid and so contributed nothing to the correction of the prevailing acidosis. It is further seen that, initially not only the absolute NAE values but also the urinary net acid concentrations were low as compared with the markedly increased NAE and NAC of the

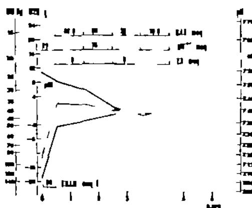


Fig 6. Course of acid-base parameters in 4-month-old male infant with culture-negative gastroenteritis and normonatremic dehydration treated with THAM.

Note The extraordinarily large NAE values in this case, due primarily to renal excretion of the organic buffer In an acid urine THAM (pK_A 7.82 at 37°C) can be demonstrated partly as titratable acid (TA) and partly as an apparent increase in the excretion of NH

4th and 5th day elicited in response to an intensified therapy Hence the rather poor renal response during the first days was not solely due to a reduced flow of urine but also involved a relative inability to excrete hydrogen ions, possibly a con

TABLE — *Renal excretion of non-volatile acid in a 3-week-old male infant with acute gastroenteritis* (Fig 3)

Period of urine collection (Fig. 3)	Diuresis (ml)	Urinary pH	NAE (mEq)	NAC (mEq/l)	NH_4 (mEq)	TA (mEq)
I	290	6.6	5.5	21.2	4.2	1.3
II	193	6.4	4.9	20.2	5.1	0.8
III	80	7.2	4.0	50 0	3.9	0.1
IV	440	5 8	20.—	45.9	17.5	2.7
V	590	6.7	34.7	38 9	32.4	2.3
VI	320	6.6	0.6	1.9	0.1	0.5
VII	480	6.8	8.1	18.0	6.7	1 4
VIII	375	6.6	9.0	24.0	6 5	2.5
IX	300	6.5	6 9	23 (	4.2	2.7
X	235	5.7	8.7	34.1	4 6	4 1

NAE Net acid excretion. NAC Urinary net acid concentration. NH — H excreted in combination with NH_3. TA Titratable acid.

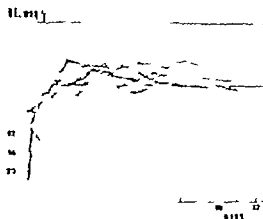


Fig. Course of base excess ( ) in 10 infants with gastroenteritis showing mild postacidotic metabolic alkalosis. For discussion, see text.

sequence of diminished glomerular filtration rate and decreased supply of sodium ions to the distal tubules. The rise in NAE during the 4th and 5th day was paralleled by an increased chloruresis (NH_4Cl) exceeding the therapeutic supply of chloride. Lack of chloride could hardly be a cause of the low NAC seen initially.

The diarrhea stopped abruptly upon resumption of intravenous fluid therapy on the 4th day and during the following 48 hours the infant passed no stools at all. During this period the BF rose from 16.4 mEq/l to 1.0 mEq/l, representing the addition of about 15 mEq of base to the extracellular compartment (calculated as 0.3 body weight in kg [37]) while the patient retained as much as 00 mEq of base (= administered bicarbonate + urinary NAE— endogenously produced non volatile acid, assumedly -8 mEq/day). The most reasonable explanation of this finding appears to be that an intracellular acidosis was being corrected along with the reduction of the extracellular surplus of non volatile acid (cf the alkalotic volume in pyloric stenosis [3*]). The rise in BE to positive values was followed by a marked decrease in NAE and a simultaneous increase in potassium excretion.

Finally Fig 3 demonstrates a slight postacidotic metabolic alkalosis lasting 3-4 days.

In 2_ infants a sufficient number of observations were made during the postacidotic phase to justify a partition in groups with and without such postacidotic alkalosis. In ten of these (4 .), a characteristic postacidotic displacement of the BE lines towards alkalotic values (Fig ") was demonstrated which could not be attributed to overdosage of bicarbonate. The postacidotic BE values are seen to settle at a level about four mEq/l above the normal mean (Fig 1) for a couple of days and finally drop to the normal range.

In seven of these infants serum Na determinations were made on admission. In five this value ranged from 15 16 mEq/l. In one infant (Fig 5) with an initial serum Na concentration of 126 mEq/l, a tentative diagnosis of transitory adrenocortical hypofunction was made while the last patient a week-old girl with Coli enteritis, presented with a serum N of 150 mEq/l. During the first 4 hours, the infants of Fig " were given on an average 0.3 mEq/kg (admission weight) of sodium (range 2 8 1) and 1.8 mEq/kg of potassium (range 0.3-4) cf discussion p. 164.

Fig 4 gives acid base values and supplementary electrolyte data of a 6-month-old male infant with culture negative gastroenteritis and hypertonic dehydration. He had been vomiting for four or five days prior to admission and had during the last 00 hours passed 15 mucous and liquid but not watery stools. On ad

mission he was tired and exhausted with doughy" skin texture and Kussmaul's respiration. There were no convulsions

It is seen that in spite of a high serum sodium level very little sodium appeared in the urine during the first four days possibly indicating that hypovolemia, rather than hypotonicity is a primary stimulus of adrenocortical aldosterone release Evidently loss of hypotonic intestinal fluid [45 57] in the presence of renal tubular sodium conservation may lead to hypertonic dehydration. The BE responded rapidly to intravenous bicarbonate therapy but during the following three days the renal NAE continued at a high rate in spite of prevailing slight post acidotic alkalosis This may indicate persisting intracellular acidosis. During the first four periods of urine collection (first 90 hours, cf Fig 4) this infant retained approximately 90 mEq of base but only half this amount (approx 46 mEq) appeared in the extracellular compartment causing a rise in BE from −*0 6 to +_4 mEq/l

The opposite situation, i.e. large urinary losses of sodium in a hyponatremic infant, is shown in Fig 5 which concerns a 3-month-old girl with Coli enteritis weighing on admission, 5.5 kg. Following a short (3 days) period of diarrhea, vomiting and lightly raised temperature, she was admitted in a condition of moderate hypotonic dehydration. She was not shocked, drank eagerly and was given Arobon[1] 5 %, orally During the first 10 hours, she passed 9 liquid but not voluminous stools and 50 ml of an acid urine (pH 5.8). The serum urea concentration rose from 94 to 148 mg/100 ml while the BE decreased from −9 9 to −14.8 mEq/l.

Twelve hours after admission, she vomited and appeared worried. The temperature rose to 39.5°C and half an hour later she was deeply shocked with dilated pupils, coolish, grey skin, and generalized convulsions. Following intratibial injection of water-soluble hydrocortisate, blood and bicarbonate a polyethylene catheter was introduced in the vein in front of the right tibial malleolus and intravenous treatment was maintained for the next four days, during which the patient remained unconscious with repeated convulsive episodes. The spinal fluid was normal.

During the latter half of the first 4-hour period, no urine appeared although about 500 ml of fluid were infused. Subsequently diuresis recurred, but in spite of a satisfactory flow of urine (3–600 ml/day) the serum urea level remained high for four days (148–150–130–"6 mg/100 ml) suggesting in retrospect a "lower nephron nephrosis" During the second 24-hour period *0.8 mEq of sodium were lost in the urine (urine sodium 61 mEq/l), while the serum Na level dropped from 1 4 to 116 mEq/l. Furthermore the initial decrease in BE seemed out of proportion to the rather small volume of intestinal fluid lost in the stools. Hence parenteral factors had to be considered, and treatment with desoxycorticosteron acetat (DOCA) and small volumes of hypertonic (10 %) N Cl was initiated. On admission, serum potassium values were normal, but subsequently hypokalemia (min. _8 mEq/l) developed.

This rather complex situation which includes initial electrolyte deficits, continued diarrhea and administration of hydrocortisate, DOCA, hypertonic saline and potassium, makes an exact evaluation

of adrenal and renal contributions to the pattern of electrolyte di turbance seen in this infant extremely difficult but it seems fairly certain that tran ient renal tubular damage was involved. Apparently insufficient renal NAE added to the acidosis during the first days and called for extrarenal correction

The patient recovered gradually and was discharged after four weeks weighing 6.2 kg One year later she appeared in excellent health apart from persisting electroencephalographic changes.

Discussion

Several factors may contribute to the metabolic acidosis regularly seen in infantile gastroenteritis

I Excessive losses of bicarbonate-basic intestinal fluids overcoming the renal compensatory mechanisms are generally held responsible [11 16 43 45]. In the normal state intra individual day variations in the acid base status [20] are quantitatively insignificant while the urinary output of net acid largely equivalates the amount of non volatile acid liberated by protein catabolism. Thus, the simultaneous effusion of HCl by the gastric mucosa and of bicarbonate by the pancreas liver and intestinal glands appears to be well balanced against the continuous absorption of bicarbonate taking place in the intestine

In achylic states, an increased intestinal bicarbonate absorption would seem to account for the maintenance of a normal acid-base status, while in excessive vomiting of gastric juice this absorption of bicarbonate is a factor in the production of metabolic alkalosis. In diarrhea bicarbonate excreted in excess of the equivalent of the gastric output of H^+ escapes absorption and acidosis ensues. In infectious gastroenteritis relative achylia may serve to augment the effect of this mechanism

Evidently the above exposition presupposes a removal from the extracellular fluid of the H^+ and HCO_3 secreted into the gastrointestinal tract If the H and HCO_3 ions are considered to be generated locally in the glandular epithelium, it becomes necessary to postulate an equivalent addition of HCO_3 or H^+ to the extracellular fluid (as in the case of the renal tubules)

The position of fecal losses of bicarbonate as a major cause of the acidosis accompanying infantile diarrhea was challenged by Teree *et al* [56] who in an analysis of 31 stools from 27 infants with diarrhea found pH values between 4 4 and 7.2 and very low bicarbonate concentrations. However as correctly pointed out by Harrison [16], bacterial fermentation of unabsorbed carbohydrate in the colon with resulting production of unknown quantities of organic acids may seriously interfere with any interpretation of rude stool data. It should be appreciated that excretion of bicarbonate (in urine or stools) from the extracellular compartment represents a loss of base irrespective of any simultaneous loss of CO_2.

II. Dehydration and hypovolemia with impaired peripheral microcirculation and tissue hypoxia might lead to accumulation of lactic acid. Several writers have emphasized this possibility [4, 5, 40], but correlations of the BE and lactate levels in infantile diarrhea have not been published. If excessive production of lactic acid were the main cause of acidosis in

infantile diarrhea with dehydration, the acidosis should be promptly reversible upon rehydration, and bicarbonate therapy would be expected to act more rapidly than lactate therapy—and should be followed by an almost equivalent postacidotic alkalosis

III In severe dehydration, decreased glomerular filtration rate, water conservation, and oliguria may seriously limit the renal NAE and so contribute to the maintenance of the acidotic state, cf. Fig 3 and Table 2. In some cases hyperkalemia may cause further retention of hydrogen ions.

IV Diarrhea, infection and therapeutic withdrawal of oral feedings may result in starvation and increased breakdown of tissue proteins with resulting rise in endogenous net acid production. This may increase the effects of an impaired renal response (III) considerably

V Starvation may further lead to ketosis, and even this mechanism of acidosis has received some attention. It has, however been rejected as quantitatively unimportant [13, 24 25].

VI With severe dehydration and shock, impaired adrenocortical function and "lower nephron nephrosis" may add to the acidosis (Fig 5) and also the possibility of cellular losses of H^+ to the plasma (e g. in response to cellular hypoxia, hypertonicity (VII), or adrenocortical hypofunction) should be given consideration. Darrow [8] maintained that the acidosis is dependent on relative excess of chloride over sodium due to transfer of sodium to the cells during dehydration. In modern terminology this would imply an exchange of cellular H^+ for extracellular Na and the resulting acidosis should be predominantly extracellular The data of the present study do not support this point of view Darrow's approach to the acid-base status in infantile diarrhea was of an indirect kind, and the balance data [9] to which Darrow referred show an excessive retention of cations (Na+K) over that of chloride—indicating, in fact, a primary loss of bicarbonate.

VII. In 196_ Sotos *et al* [54] in experiments with rabbits, showed that intravenous infusions of hypertonic solutions of NaCl, sucrose or urea gave rise to a metabolic acidosis which was strongly correlated to the degree of extracellular hyperosmolarity achieved and which could not be explained as a dilution effect. The osmolarities at which significant acidosis occurred were of an order of magnitude which may well be encountered in infantile hypertonic dehydration. Sotos *et al* attributed the acidosis to a transport of hydrogen ions from the cells to the extracellular fluid.

It was not possible in the present study to establish a convincing clinical analogue to the experimental findings of Sotos *et al* The very slight negative correlation between the BE and serum Na values of Fig. 2 is more likely due to the fact that while high serum Na values are always indicative of rather severe and protracted loss of intestinal fluids, normal values offer no clue to the severity of the illness or the state of hydration. However these data do not exclude a contribution of hypertonicity to the acidosis in cases with severe hypertonic dehydration, and the possible association between postacidotic alkalosis and hypertonicity (p 160) as well as the apparent difference in distribution of the total base deficit observed between the

cases illustrated in Fig 3 and 4 might be interpreted in favour of this possibility But again, if hypertonicity were a main cause of acidosis in some cases, this acidosis should be predominantly extracellular and the gross estimate of total base retention by the infant of Fig 4 definitely suggests the existence of an intracellular acidosis Further studies are clearly needed

More than forty years ago Friderichsen [1-] using an equilibration technique studied infantile acidosis in terms of the "reduced hydrogen value" (now pH_{40}) calculated according to Hasselbalch [22] a quantity comparable to the standard bicarbonate of Astrup [27], and he was the first to recognize the phenomenon of postacidotic alkalosis in infantile gastroenteritis. Friderichsen considered this alkalosis to be due to biological overcompensation In fact the persistence of a mild degree of metabolic acidosis through several days (Fig 7) might suggest that during this period the neutrality regulating functions were aiming at a higher BE level Possibly a secondary adrenal cortical hyperactivity induced by the stress of infection, dehydration, and acidosis might persist long enough to manifest itself in a slight postacidotic alkalosis

An alternative and perhaps more likely explanation of the postacidotic alkalosis is that it may be due to protracted potassium deficiency It has been known for some time that the administration of sodium to potassium deficient patients and animals may lead to aggravation of potassium depletion and alkalosis [3 8, 20 36]. Cheek [3] compared groups of infants recovering from acidosis due to gastroenteritis. Seventy two hours after admission infants with a high sodium intake (11-25 mEq/kg admission weight) during the first 24 hours (group I) consistently showed metabolic alkalosis, while infants receiving low (0-7 mEq/kg) initial sodium loads (group II) did not Both groups were given 3 mEq/kg of potassium in the first 24 hour period Conversion of the data of Cheek by means of the alignment nomogram of Siggaard Andersen [51] shows that this postacidotic alkalosis was of quite the same order of magnitude as that shown in Fig 7 of the present paper While the initial sodium intake of the infants of Fig 7 correspond to the low sodium load of Cheek's group II the ratio between initial doses of Na and K is more similar to that of Cheek's group I At any rate the quantity of potassium given, in the first 24 hours, to the patients of the present series is probably well below optimum. In the large series reported on by Schlesinger *et al* [48] postacidotic alkalosis was a frequent finding a few days following admission, and also in these infants potassium deficiency may have been responsible

As mentioned above several authors have claimed pathogenetic significance of accumulated organic acids to the development of acidosis in infantile gastroenteritis, and postacidotic alkalosis in bicarbonate-treated patients might be taken as indirect evidence in support of this assumption Following neutralization of the surplus non volatile acid by bicarbonate oxygenation of the organic anions might well give rise to a transitory metabolic alkalosis However the infants described by Friderichsen [1] received no base therapy and the rather uniform level of the postacidotic BE lines (Fig 7) in infants with different degrees of initial acidosis receiving

different amounts of bicarbonate is equally inconsistent with this hypothesis.

Parenteral base therapy in diarrheic acidosis was made widely known through the works of Hartmann [18 21] and was introduced in this country by Kirk [34]. Somewhat discouraging results of early workers were later attributed to failure to repair the accompanying potassium deficit, and for some years base therapy in infantile gastroenteritis was to some extent discredited [3 8].

However renal correction of severe metabolic acidosis is a slow process requiring, as a rule several days and is, furthermore dependent on proper rehydration and repair of existing electrolyte deficits. It appears to the author that there exists no good reason to withhold initial base therapy in the management of infantile diarrhea with acidosis. On the contrary such therapy may considerably improve the chances of survival. Recent years have brought new and more precise information concerning many aspects of the physiology of the acidotic state. Under various experimental and clinical conditions, the following changes have been related to a decreased pH. "Transmineralization with cellular losses of phosphate [47 50] and potassium [28 50], hemoconcentration [7], pronounced increase in the plasma catecholamine level [7 41]—perhaps in consequence of a decreased vascular [.. 7] and cardiac [7] responsiveness, decreased amplitude of left ventricular contraction and reduced coronary flow [4..], increased adrenocortical release of hydrocortisone [38], decreased plasma protein binding of thyroid hormones [16], and inhibition of the cellular lactic dehydrogenase system [50]. These findings, and in particular the evidence of progressing circulatory impairment with low pH values, serve to emphasize the importance of early correction of severe acidosis. As regards the acidosis of infantile diarrhea rehydration, initial correction of the acidosis under close laboratory monition and early administration of potassium according to current principles would appear to be the treatment of choice [53]. In the management of acidosis with normo- or hyperosmolar dehydration, THAM [40 50] may prove valuable but the clinical use of this amine buffer awaits further exploration of its pharmacological properties and toxicity

Summary and Conclusions

Severe or moderate metabolic acidosis was demonstrated in 34.3 % of 70 consecutive cases of acute gastroenteritis studied (normal values are supplied) The acidosis appears to be primarily due to intestinal losses of bicarbonate but several factors (e.g. increased protein catabolism, renal and adrenal hypofunction, tissue hypoxia) may contribute The acidosis appears not to be confined to the extracellular compartment Mild postacidotic metabolic alkalosis, probably due to protracted potassium deficiency (i e deficient initial supply of potassium), was noted in several instances. Early correction of severe acidosis by parenteral administration of base should not be neglected. The Astrup micro method provides a useful tool in the monition of such therapy

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Department of Pediatrics
Odense Amt and Bys Sygehus
Odense
Denmark

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CASE REPORTS

A New Type of Meningo-Encephalitis Associated with a Rhinovirus

by A HOLZEL, PATRICIA A. SMITH and J O H TOBIN

From the Booth Hall Children Hospital and Public Health Laboratory, Manchester

A large number of viruses have been shown to cause aseptic meningitis since Wallgren [6] first described this syndrome but so far rhinoviruses have not been included among the causal agents. During 1964 we observed three children with an unusual form of meningo-encephalitis which may have been due to infection with an agent of this group

Clinical Findings

Case 1 (D T)

A 1 -month-old female infant was perfectly well until three days before admission to hospital on 25 v 64, when she started vomiting refused her feeds, became listless and almost motionless.

Past history The baby weighed 2380 g at birth and her mother pregnancy and delivery were normal The infant was receiving a weaning diet and was developing normally Her older sister and her parents were healthy and without any record of recent illness.

On examination the baby was pale semi comatose and responded only to painful stimuli. Her rectal temperature was 39°C her pulse rate 128 and respiration rat 4 per minute The anterior fontanelle was bulging there was some neck rigidity and Kernig and Brudzinski's sign were posit e. Her plantar responses were flexor he tendon reflexes diminished and the tone of the lower limbs slightly increased. The pupils were contracted and reacted sluggishly to light *The liver and spleen were palpable two* fingerbreadths below the costal margin. No other abnormalities were detected t that time Her head circumference was 43.1 cm.

A few hours after admission the infant developed a series of convulsions which responded temporarily to phenobarbitone but continued ff and on for the next 48 hours. During this time ophthalmoscopy revealed extensive retinal haemorrhages, some close to vessels, others in areas away from them. With the cessation of convulsions the temperature returned to normal but the neck rigidity persisted and the fontanelle remained tense for some days. A fortnight later the retinal haemorrhages were still present but had disappeared when examined six weeks after the onset of illness. Although the child a general condition was satisfactory it was suspected that she might ha suf fered some cerebral damage however when re-examined t 1 months of age she was found to be well and progressing normally Her hearing and vision seemed normal except for an alternating strabismus. The head circumference was only 44 4 cm. During her stay in hospital repeated Mantoux tests (1/1000) were negative as were X-ray examination of her skull heart and lungs.

Laboratory investigations

C.S.F 25.v.64 20 cells (90 % lymphocytes)/mm protein 30 mg % sugar 56 mg % chlorides 00 mg %

C.S.F 8.vi.62. 2 leucocytes/mm 416 RBC/mm protein 30 mg % sugar 69 mg % chlorides 750 mg ,, globulin—no increase.
Haemoglobin 25.v.62. 8.4 g/100 ml.
White cell count (1) 25.v 62. 22,600/mm (N 69 %, L 28 % M 3 ,,). (2) 29 v 62. 41,300/mm (N.3* % L. 58 %, M. 9 %)
Platelets 293,000/mm³ *ESR* (Micro method) 35 mm in one hour

Bacteriological examination of the nose and throat on three occasions gave a moderate growth of normal flora.

Case * (*G I*)

A six month-old male infant had been in good health until two days before admission on 18.x.62 when he received his first injection of triple vaccine (D.P T). Four hours later he began t whimper became very restless, but continued t take his food for the next 24 hours, when he started vomiting and a few urticarial wheals appeared on one rm. After a relatively quiet night the infant became very hot, vomiting recurred, and he had several short convulsions, with twitching of the face and limbs.

Past history The baby had been born at full term after a normal pregnancy and deli very and was developing satisfactorily He was the fifth child of healthy parents and there was no history of recent illness among his family

O examination the patient looked very ill, with his eyes rolling from side t side His fontanelle was bulging his neck stiff Brudzinski sign was strongly positive but Kernig was negative His pupils were equal and reacted to light. The optic discs showed slight temporal blurring and the retinal eins were congested. His tendon reflexes and plantar responses were normal. No further abnormalities could be found in the other systems. His temperature was 39 7°C and pulse rat 160 per minut During the first day in hospital the baby developed a fine petechial rash on his arms, legs and trunk, and this lasted for 24 hours. H became comatose his temperature rose to 40.5°C and he had series of minor fits. His bdomen became distended and he vomited some altered blood. Antipyretic measures were successful in gradually reducing his temperature with a concurrent improvement in the baby general condition. The convulsions stopped and consciousness returned slowly over a period of a few days. After one week a small area of redness was found temporal to the left disc and two days later extensive haemorrhages in both retinae similar to those seen in Case 1 A slow nystagmus also appeared at this time During the next three weeks the patient recovered, the retinal haemorrhages cleared but the nystagmus persisted and was still present ten months later At this time the infant could sit up without support had started to crawl, but was still unable to walk or talk. His E.E.G was normal and his head circumference 46.3 cm. It seemed that the child s development had been delayed by his illness, but there was no gross mental retardation.

Laboratory vestigations

C.S.F 18.x.62. 217 cells (70 % polymorphs)/mm protein 35 mg %, sugar 5 mg %, chlorides 740 mg ,,
C.S.F 22.x.62. 4 leucocytes (mononuclear)/mm³ RBC 45/mm³ protein 20 mg/100 ml, sugar 62 mg/100 ml chlorides 730 mg/100 ml, globulin—n increase Culture—no growth.
Haemoglobin 8.4 g/100 ml. Blood group A Rh. neg
White cell count 18.x 62 17,000/mm (N 62 %, L. 34 % M. 1 % Turk cells 3 %).
Platelets 294,000/mm³ ESR (Micro method) 50 mm in one hour 25.x.62. Total serum proteins 5.8 g/100 ml, serum albumin ..6 g/100 ml, serum globulin 3.2 g/100 ml.
Album /globulin 0.8. α globulin 0.37 g/100 ml, α_2 globulin 0.49 g/100 ml, β globulin 1 17 g/100 ml, γ globulin 1 17 g/100 ml

Bacteriological examination of the throat and stools produced no pathogens and a blood culture was negative.

Case 3 (D. C.)

A twenty nine month-old infant was admitted on 6.xii.62 to a peripheral hospital with a three weeks history of listlessness, anorexia, frequent vomiting and bronchitis which had been treated by the family doctor with penicillin.

Past history. H. had been delivered by caesarean section at full term. His birth weight was 3915 g. The neonatal period was uneventful and he seemed to have developed normally except for some delay in talking. H. had no severe illness previously.

When first seen he appeared irritable pale and listless. The head circumference was 52 cm. The pupils were equal and reacted to light. The fundi and cranial nerves were normal. There was no neck rigidity and he could move his limbs freely though some degree of ataxia was noticeable. The muscle tone of all the limbs was considerably reduced. His consciousness was clouded but he responded when called by his name. At times he made grasping efforts with his hands when objects were placed before him. Tendon reflexes were brisk. Radiological examination revealed a cervical spina bifida occulta. In the course of the next two days he developed a mild left hemiparesis, retinal changes became visible which could not be readily interpreted and as the lumbar puncture revealed a polymorphonuclear pleocytosis he was transferred to a neurosurgical unit on 8.xii.62.

There the presenting signs were still the extreme lethargy and irritability. His dilated pupils did not react to any but the strongest light and then only minimally. The fundi showed early papilloedema on the left side and small haemorrhages throughout both retinae; a third of these seemed to have a yellowish whitish centre lying in or on the wall of a vessel. Many of the haemorrhages were not in the vicinity of either vessel or disc. The macular areas remained clear.

The muscular hypotonia had increased, so that he was unable to support his head and the ataxia of the upper extremities was more striking. The tendon reflexes were still brisk, the abdominal reflexes absent and both plantar reflexes extensor. During the following five days drowsiness persisted and vomiting recurred frequently. A generalised convulsion was controlled by 2 ml of paraldehyde intramuscularly. Two hours later he suddenly collapsed with periods of apnoea and irregular respirations interchanging. The increased papilloedema indicated a further rise in intracranial pressure which was relieved by ventricular tap and release of 40 ml of clear C.S.F., after which the patient regained consciousness. Ventriculography showed dilatation of the lateral and third ventricles and changes in the skull wall below the torcular suggestive of an intracranial dermoid cyst. Craniotomy was carried out and a large thin walled cyst removed containing pus, sebaceous material and hair.

The postoperative period was rather stormy and there was only a very slow improvement. One month after operation the child was discharged home. Recovery continued at home though ataxia in the upper limbs and particularly in the left arm remained obvious. However two months after his return home signs of increased intracranial pressure became manifest again and a ventriculogram showed a greatly dilated right ventricle. The patient was readmitted to hospital for the insertion of a low pressure Holter valve and subsequently made good progress.

At his last attendance in the outpatient department a year after his first admission he appeared to walk normally, use his hands without signs of ataxia or tremor and his speech was developing at a satisfactory rate. Findings on neurological examination were normal.

Laboratory investigations

Blood urea: 15 mg/100 ml.

Blood group: O Rh (D) pos.

Urine: Nothing abnormal detected.

C.S.F. 6.xii.62. L.P. 40 cells (75% polymorphs)/mm³, protein 33 mg %, sugar 50 mg %, chlorides 50 mg %, no excess of globulin.

C.S.F. 11.xii.62. L.P. 12 lymphocytes/mm³, 6 RBC/mm³, protein 50 mg %, Pandy's

test—faintly positive Culture—no growth. Gram staining—no organisms seen.

C.S.F 20.xii.62 From right and left ventricles. Clear colourless. 2 lymphocytes/mm³ protein 10 mg % glucose 78 mg chlorides 695 mg % Pandy's test—negative

Blood 6.xii 62 Haemoglobin 76 White cell count 12,700/mm³ (N 78 %, L 17 M. 5 %) Blood culture negative.

E.E.G 15.xii.62. The resting record showed high voltage theta and delta activity dominant in all areas throughout the record. Conclusions: gross generalized abnormalities but no focal or abnormal paroxysmal discharge The findings are non specific but would suggest widespread disturbance of cerebral function.

31.xii.62. *Toxoplasma*. Dye test <1/8 and Complement fixation test <1/4. Pus from cyst Gram, numerous degenerat pus cells. Gram-positive cocci in pair and clusters, Gram positive short fat and filamentous bacilli, Gram negative bacilli. Primary culture—no growth; after enrichment —no growth.

Cyst from bra The histological picture was that of an infected and partially ruptured dermoid cyst with chronic inflammatory reaction, fibrosis and numerous macrophages.

Virus investigations

During 1962 and 1963 the majority of children entering Booth Hall Hospital had throat swabs taken in the admission room when first seen. The swab was used to inoculate one secondary rhesus monkey kidney cell culture and one HeLa cell culture which were then held at 33–34°C in roller drum, this apparatus being kept in an incubator in the admission room (Holzel *et al* [3]) From such a routine throat swab taken from Case 2 (G I.) on the third day of illness, a cytopathic agent was isolated in the monkey kidney cell culture The agent (G.I. virus) produced an enterovirus type of cell degeneration in the monkey kidney cells. This change was produced appreciably better when incubated at 33–34°C than at 37°C when cultures were rolled rather than left stationary and when the bicarbonate concentration was kept below 0 08 %. Although some cytopathic change could be produced under normal conditions, it appeared much quicker and to a higher titre when "common cold" conditions (Taylor Robinson & Tyrrell [5]) were employed. The agent was not neutralized by antisera against the poliomyelitis and Coxsackie B viruses, by Coxsackie A7 or A9 antisera or by antisera to ECHO viruses types 1 to 28 or Frater virus (ECHO type 30) It was acid-sensitive being destroyed at pH 4 in 3–4 hours (Ketler *et al* [4]) The agent was resistant to ether did not haemagglutinate human O" guinea pig or fowl erythrocytes, or fix complement with adenovirus antisera. It did not produce a cytopathic effect in primary human amnion cultures (Hayashi & Logrippo [ ]) but grew well in the WI 38 line of human embryo lung cells. It produced a partial cytopathic effect in "Bristol" HeLa cell cultures maintained in 0.3% lactalbumin in Hanks solution containing 0.08 % bicarbonate and .5 % embryo calf serum. This partial cytopathic effect did not alter on repeated passage. The maximum titre obtained in monkey kidney cell cultures was 10^4TCID$_{50}$ per ml. Its growth in monkey kidney cells was not affected by a concentration of 190 μM 2 (hydroxyl benzyl)-benzimidazole (HBB) per ml in contrast to local isolates of ECHO viruses types 2 6 14 16 20 .3 and of Coxsackie viruses types B1–4 which were all inhi

lited by this amount Two rhinoviruses H G P and S.N which were used as controls, were also resistant to this concentration of HBB (Eggers & Tamm (1]) The agent passed through a gradocol membrane with an average pore diameter of 50 mμ These characteristics would indicate that the virus (G I) was an M strain of rhinovirus. No cytopathic agent was isolated from samples of stools taken from this patient during the second week of his illness. No complement fixing antibody to mumps or adenovirus was found in his or the other patients sera

No virus was isolated from throat swabs taken either from Case 1 during the second and third weeks of her illness or from Case 3 during the fourth and fifth weeks of his illness. No virus was found in repeated faecal samples taken from either patient during the same periods.

Neutralising antibody studies with G I virus were done on the sera from the three patients and from a small group of normal subjects Four fold dilutions of the patients sera (1/4 to 1/4000) were mixed with an equal volume (0 0 ml) of a G I virus suspension containing approximately 100 $TCID_{50}$ and held at 37° C for 45 minutes. Zero point five ml of each virus/serum mixture was added to each of two rhesus monkey kidney cell cultures and incubated at 33 C, the tubes being examined at three and seven days. The 50 end point of neutralization was used to express the serum antibody level. A similar method was used for titrating sera from normal subjects but only one monkey kidney cell culture was used for each serum dilution/virus mixture The serum titres for these latter subjects were given as the highest dilution inhibiting virus growth The antibody titres found in the patients are given in Table 1 and for the other subjects in Table 2

TABLE 1 *Neutralizing antibody titres to G I virus*

	Serum sample		
Case	1	2	3
1	1/16 (9)*	1/1024 (16)	—
	1/8 (6)	1/256 (23)	—
3	1/1[illegible] (1)	1/32 (41)	1/1024 ([illegible])

* Days after onset of illness.

TABLE 2 *Neutralizing antibody titres to G I virus in normal subjects*

	Age			
Antibody titre	4 mths	4 12 mths	1-6 yrs	> 20 yrs
1/1024	—		—	2
1/256	—	1	—	2
1/64	—		1	4
1/16		1	—	2
1/4	3	—	—	2
< 1/4	4	9	5	1
Total	9	13	6	11

Discussion

As the syndrome described here produced changes in the retina as well as in the central nervous system, we have called it Retino-Meningo-Encephalitis.

The onset of the illness was either acute or subacute vomiting listlessness, convulsions, coma of varying duration and ocular signs being the characteristic clinical features. The most striking eye change was a form of retinopathy associated with haemorrhages of varying extent Temporary loss of the pupillary reflex occurred in two patients and horizontal nystagmus in the third Resolution of the retinal haemorr

hages took from 3–6 weeks Increased intracranial pressure was a feature of all cases and although neck rigidity did occur it varied a great deal in severity After the acute phase the E.E.G did not show any gross abnormality and there was no recurrence of the convulsions during the recovery period. In one patient immunisation with triple antigen seemed to act as a precipitating factor as did an infection of an intracranial dermoid cyst in the patient with a cervical spina bifida occulta

In the latter infant (Case 3) there was a disconcerting overlap of manifestations due to two disease processes affecting the central nervous system simultaneously However it would seem that the initial phase of the illness was the result of a virus infection, since the C.S F pleocytosis of 40 cells, which at first consisted largely of polymorpho-nuclear cells, fell to 12 cells (all lymphocytes) within a week and returned to normal in the course of a further ten days. During this period the retinal haemorrhages, which we also regard as attributable to the virus infection, regressed, whilst the papilloedema, and other signs of raised intracranial pressure due to the infected cyst increased.

The total white cell count was raised in all cases, as was the erythrocyte sedimentation rate in the two cases in whom it was determined during the initial stage The cerebrospinal fluid showed some degree of pleocytosis in all the patients

A rhinovirus was isolated from the throat of the only case tested in the first week of the disease but all patients showed som rise in nt'body to this virus during their illness. The rise in two patients indicated concurrent infection with the G I virus during their illness while in the third, who was not first bled until the end of the third week of illness the rise was suggestive of recent infection with this agent Another rhinovirus (S.X strain) serologically distinct from G.I. virus was isolated in 196_ from the throat of a two-week old baby who died shortly afterwards from encephalitis and the relation of these two agents to other cases of meningo-encephalitis occurring since is being investigated. It is not surprising that a rhinovirus could cause occasionally a more serious disease than a common cold as it would merely be following the pattern of many other virus infections.

Because of the multiple sero-types the isolation of virus is essential if the necessary antibody studies on cases of meningo-encephalitis are to be pursued so that the role of rhinoviruses in this syndrome can be confirmed.

Unfortunately nose and throat swabs must be taken early in the disease for successful isolations to be obtained. Both of the rhinoviruses isolated were from specimens taken routinely and not from those taken because of the patient s disease It is of interest that the only two strains of hinovirus isolated during 1962–63 from over 2,000 children admitted to Booth Hall Hospital with respiratory and other diseases, and investigated for virus infection, were both from cases of central nervous system disease

Antibodies to G I virus at various concentrations were present in thirteen of fourteen adult women, but only in seven of twenty-one children, aged from four months to six years. This would suggest that although this virus was present in 196_ it may not have been bout for a few years in the Manchester area. The low

content of antibody found in the infants under four months was considered to be transmitted maternal antibody rather than their own (Table 2).

Although absolute proof of the aetiological role of the rhinovirus isolated in causing retino-meningo-encephalitis is absent, the concurrent infection with it may be more than a coincidence.

Summary

A hitherto unrecognised form of meningo-encephalitis associated with retinal changes has been described in three patient. It was associated with concurrent infection by a rhinovirus (M strain) isolated from the throat of one of them, and it is possible that this virus was the causal agent. Antibodies to this virus were also found in a number of healthy subjects.

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Manchester
England

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CASE REPORT

Congenital Type of Generalized Lipodystrophy

by KONSTANTIN B. CHOREMIS, BASIL CONSTANTINIDES and CHRISTOS A. KATTAMIS

From The Department of Pediatrics University of Athens. Director Professor K. Choremis

Since 1959 Seip has described five patients with a bizarre syndrome, of obscure etiology characterized by generalized lipodystrophy increased growth rate and acromegaloid pattern large hands and feet advanced bone age and dental development muscular hypertrophy increased muscular glycogen, hepatomegaly corneal opacities, skin pigmentation dry coarse skin, hypertrichosis and phlebomegaly [11 L.]

In the literature the syndrome is referred under a variety of titles as, undiagnosed endocrinometabolic syndrome [1] lipodystrophy and gigantism with associated endocrine manifestation [11], lipodystrophic muscular hypertrophy [10] generalized lipodystrophy [13], congenital muscular hypertrophy [6], "hypertrophie musculaire généralisée à début précoce avec lipodystrophy faciale" [6].

The clinical and laboratory findings in a young infant with the congenital type of this rare disorder are reported in this paper

Case Report

The patient S Ch. was the first child of normal parents with no consanguinity She was admitted to the Department of Pediatrics of the University of Athens at the age of 10 months. Pregnancy had been uneventful, delivery normal and birth weight 3280 g Family history was negative. Appearance at birth was described as "peculiar"; she was thin and bony lacked subcutaneous fat and had a wrinkled face She gained weight normally She sat at the age of 6 months and had the first tooth at about the same time. The rate of growth was accelerated and she had muscular hypertrophy with increased muscle power

On physical examination the height was 78 cm, the skull circumference 42 cm and the weight 9450 g The subcutaneous fat was lacking over the entire body and emaciation was prominent. Because of the paucity of facial fat the patient had a progeroid appearance. The abdomen was markedly protuberant the umbilicus everted and the abdominal muscles visible Superficial veins were prominent especially at the extremities (Fig 1); there was marked generalized hypertrophy of the muscles, with increase of their power Reflexes were normal. The patient showed acromegaloid pattern; she was tall, with large feet and hands and macrognathia (Fig 2) Hirsutism was also present more pronounced over the extremities and on the back. The scalp was covered by abundant, thick curly hairs. Labia majora and clitoris were moderately hypertrophic. No pubic or axillary hairs were present The liver was palpable A grade II blowing systo-



Fig 1 The patient t 10 months.



Fig .. The patient at 20 months.

he murmur wa lso heard at about the 5th intercostal space by the left sternal border; blood pressure wa increased, 130 80 mm/Hg

Laboratory Data

The urine was normal chemicall and microscopically; chromatography revealed a light increase of amino acid excretion

The blood examination showed Hb – 11.3 g %, WBC 10,000 per mm (neutrophils *0 .. lymphocytes 80 platelet in normal numbers, red cell morphology normal) ESR first hour 10 mm.

Blood urea, 1 mg/100 ml Blood sugar 88 mg per 100 ml

Serum proteins Total, 7.8 g per 100 ml (albumin 5.1 g globulin .. g) P per Electrophoresis: Albumin 50 .. globulins α – 7 1 %, β – 7 1 β 3.3 .., and γ 33 %.

Serum chloride 110 mEq/L, serum sodium, 132 mEq/L, serum potassium, 5.. mEq/L serum calcium, 10.5 mg/100 ml, serum inorganic phosphorus 3.2 mg/100 ml. Alkaline phosphatase 4.5 Bodansky units. Total cholesterol **0 mg/100 ml. Total lipids 91 mg/100 ml.

Blood lipograms Fraction – 22.6 % # 45 and β 36.4 ..

Liver function tests Thymol turbidity 4.3 units, zinc sulfate turbidity 4.7 units, cephalin-cholesterol flocculation +

Repeated est mation of fasting blood sugar ranged from 88–115 mg/100 ml. Following the ingestion of glucose (2 5 g per kg body weight) the blood sugar concentration rose from fasting value of 115 mg/100 ml to 1.., 138, 117 1 4 mg/100 ml in 30 60, 120 and 180 minutes.

Urinary neutral 17 ketosteroids were 3.1 mg. pregnanediol 0 03 mg, dehydro-epi-andro-



Fig. 3. The sclerotic skeleton; advanced bone age at 10 months.

sterone 0.6 mg/24 hours. Creatinine excretion was 88 mg/kg/24 hours.
The basal met bolic rat was increased (+30).
ECG and EEG were normal. In opthalmoscopic examination nothing bnormal was de tected
Sl biopsy Sex chromatin was positive (Female) Subcutaneous fat was absent and mild edema of the eborium was noted.
Roentgenographic examination Radiographs demonstrated diffuse enlargement of the heart; the skull, vertebrae and extremities seemed sclerot w th increased calcium con tent (Fig 3). An advance in osseou development corresponding t the age of 3 years, w also noted. Intravenous pyelography as normal; barium meal disclosed typical picture of dolichosigmoid.
The intelligence quotient was within normal limit (I Q 90)
Cl ical Course The patient was followed up regularly t 6 months intervals. She now 30 months old, her height is 93 cm and weight 14 kg She walked t the age f 13 months. She is very active but irrit ble The emacia tion, the progeroid facies and the muscular hypertrophy are now more pronounced.
At the age of 2 years her bone age corresponded t that of a child of 4 years. Laboratory investigations were rather identical t the previous ones, except that holesterol was found t be 257 mg/100 ml total lipids 873 mg and 778 mg the total proteins 6.5 g and liver function test showed mild impairment (thymol turbidity – 10 unit) The blood pressure was increased t 160/95 mm Hg, and liver was palpable 4–5 cm below the costal margin.
F ther blood was examined for total lipids and were found t be 600 mg per 100 ml.

Discussion

Only a limited number of patients with identical or closely resembling cl nical picture have been previously reported Lipodystrophy is the common feature of

the acquired and the congenital group Most cases of the acquired form have been described in adults and were associated with diabetes [^ 7 9]. The clinical features and biochemical disturbances of the congenital group have been described in detail by Seip [11 1]. Lipodystrophy is generalized and is not restricted to subcutaneous tissues, but affects also the mesenteric and perirenal fat [10] The absence of fat is responsible for the appearance of the face enlargement of the abdomen and phlebomegaly

In our case lipodystrophy was associated with the most of the clinical signs of the syndrome such as, muscular hypertrophy advanced osseous development acceleration of growth with gigantism and acromegaloid pattern (large hands and feet coarse face features thick lips and macrognathia) moderate hypertrophy of external genitalia without signs of sexual precocity pigmentation hirsutism with abundant thick and curly hair of the scalp, enlargement of the liver and cardiomegaly with elevation of blood pressure

} Corneal opacities were not present and pneumoencephalography which may disclose hypothalamic lesions [11] was not performed.

There is a good evidence suggesting that some cases of the congenital group are hereditary with a recessive mode of transmission with full clinical expression in the homozygous state and a disturbance of lipid metabolism in heterozygous one This is supported by the fact that in affected families, a) consanguinity is frequent b) a second child may have the disease and c) the parents have some degree of hyperlipemia with a similar pattern to that of the patients.

In our case the family history was negative

The clinical and laboratory findings of that syndrome strongly suggest a hormonal basis that can be attributed to hyperfunction of the anterior pituitary gland, with simultaneous overproduction of at least four hormones namely STH ACTH, MSH, and adipokinin

The hyperexcretion of somatotropic hormone may be responsible for acromegaloid gigantism and muscular hypertrophy adipokinin for lipodystrophy hyperlipemia and fatty infiltration of the liver ACTH for advanced bone age hypertrichosis genital hypertrophy and high 17 ketosteroid output and MSH for hyperpigmentation [15]

As has already been pointed out pneumoencephalography has disclosed abnormalities suggesting the presence of hypothalamic lesions [11 1°] It is reasonable to assume that a causative relationship may exist between these lesions and anterior pituitary hyperactivity This hypothesis was advanced by Seip [11] and is further supported by the findings of postmortem examination of one case that showed lesions in the floor of the third ventricle hyperplasia of the basophil cells of the anterior lobe of the pituitary gland and adrenocortical hyperplasia [1].

Furthermore the evidence that the congenital group may have a genetic basis, justifies the opinion of Seip that the whole syndrome may be the result of congenital hyperpituitarism of hypothalamic origin.

Summary

A girl 30 months old, with a number of peculiar features including lipodystrophy acromegaloid gigantism muscular hyper-

trophy hirsutism, hypertrophic genitalia enlargement of the liver advanced osseous development disturbances of lipid and carbohydrate metabolism as well as increased 17 ketosteroid excretion and basal metabolism, is described. Most of these features were present since birth. The whole clinical and laboratory data indicate that this case is identical to the congenital form of generalized lipodystrophy There is strong evidence suggesting that the syndrome results from hyperactivity of the pituitary gland and overproduction of STH adipokinin ACTH and MSH. The existence of hypothalamic lesions may play a primary role in the hyperfunction of pituitary gland. It is possible that the condition in some cases is inherited as a recessive autosomal disease

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Department of Pediatrics
St. Sophie Children Hospital
Athens
Greece

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REVIEW ARTICLE

ACTH and Corticosteroid Treatment of Infantile Spasms with Hypsarhythmia

by B. HELLSTRÖM and E. OBERGER

Since the first report by Sorel in 1958 on the remarkable effect of ACTH in the control of minor motor seizures many investigations on this therapeutic problem have appeared (1–2.) The anti-convulsive effect of hormone therapy has been generally confirmed as has its ability to reduce or convert to normal the intense and chaotic EEG abnormalities. However some of the first enthusiastic reports of the treatment's beneficial effects on psychomotor development have not been confirmed. This has cast some doubt on the ultimate value of hormone treatment but nevertheless some investigators have found cases in which the improvement in mental state has been remarkable and lasting. They conclude that in this respect also the treatment can be of great value. Because no more effective treatment exists for this group of severe epileptics, the general feeling is that a therapeutic trial with hormones should be made.

Most published series have been too small to establish which factors influence the results of therapy. Recurrence after treatment has been discontinued is a common finding and the follow up periods have been too short in many studies. However different statements about the incidence of relapses make it difficult to establish definite conclusions on this point.

Infantile spasms with hypsarhythmia are epileptic manifestations that can occur in diseases of widely different etiologies. This heterogeneity makes it difficult to compare different reports and may be responsible in part for the differing therapeutic results. There is a trend to find better results in idiopathic epilepsy whereas the well-defined, degenerative heredo-familial diseases have a poor prognosis. The latter constitute only a small proportion of all cases of infantile spasms.

Among other factors possibly correlated with results has been the time lapse between the onset of symptoms and the initiation of therapy. A short lapse has usually been associated with better results but this relationship has not been consistently reported.

A Swedish cooperative study on the effect of ACTH in the treatment of infantile spasms has dealt with some of these conflicting points and will be published elsewhere. A comparatively large number of cases has been treated in this study. Also a review has been made of all the available literature cited in the Index Medicus through December 1963. This review together with some of the data from the Swedish cooperative study forms the basis of this report which analyzes the influence of some of the previously men-

tioned variables on the early results of hormone treatment. However when evaluating these figures, it is important to recall that the report have been extremely heterogeneous. Important data are lacking in many case histories and different means of diagnostic work-up have often been employed Different classifications and evaluations, and different methods of treatment and periods of follow up examination further complicate the findings. Pooling such heterogeneous data can at best only disclose general trends. For this reason no statistical evaluation of the pooled material has been made

Material and Methods

The review covers four hundred and seventy cases in which the clinical description of the seizures was that of infantile spasms. The clinical picture was generally characterized by repetitive, symmetrical, propulsive fits of short duration. In most of the cases the disease had started in infancy and the EEG corresponded t the description of hypsarhythmia. Findings from an initial EEG examination were vailable in four hundred and thirty two cases. Reasonably adequat information bout psychomotor development in t least some stage of the disease had been obtained in three hundred cases.

The early optimal results of treatment achieved during the initial course of therapy ha been recorded in this material. When vailable data have also been collected about the persistence of the results in the first month following termination of drug therapy This has made it possible t esti mat the number f recurrences after treatment had been stopped.

The next ariable analyzed has been the etiology Here the cases ha been di ided int two groups (1) etiology known or probable and (2) etiology unknown. In this respect a known etiology means only that brain damage prior t the onset of seizures had occurred, but there has generally been no reason t believe that this injury had in itself progressed in a manner similar t the progression characteristic of infantile spasms with resultant mental deterioration in untreated cases. In this group of "known" etiology other signs of brain damage such as cerebral palsy or slow psychomotor development before the onset of the seizure disorder have been frequent. In the group of "unknown" etiology cases appear where the infant has been entirely normal in all respects until the onset of seizures. In the latter group only cases have been included where it was positively st ted that no potential brain injury had occurred. In many cases the data have been inadequate and in still other cases the significance of the presumed etiological factor has been very doubtful. Classification into one of these two groups has accordingly been possible in less than half of the cases, i.e. in two hundred and six patients. Furthermore, the figures given refer only t the early optimal results of treatment N attempt has been made to analyze the results or analyze possible differences after a more prolonged follow-up.

Concerning the lapse f time between onset of symptoms and initiation of therapy two limits were arbitrarily hosen. the first t one month and the other at three months. Data were available in one hundred and eighty-one cases t allow grouping within the one month period. This ga e a smaller group treated within one month and a larger one treated after that period. Within the three month period the grouping could be done in two hundred forty cases, with ninetythree cases being treated within three months.

The bove f gures for the different groups refer only to those cases with convulsions. EEG data were vailable for most of them, whereas an accept ble evaluation of psychomotor development and it course during therapy were reported less regularly

Results

The results are given i Tables 1–4 and Fig. 1–4 Concerning the earl optimal

results it is seen from Table 1 and Fig. 1 that convulsions and EEG abnormalities disappeared or were reduced in approximately 70 % of the cases. In contrast a favorable effect on psychomotor development was claimed in only approximately 30 % and in only one-third of these (i.e. in 10 % of all the cases) had psychomotor development returned to normal. Clinical data especially concerning EEG findings and psychomotor development are available in a minority of the cases one month after the termination of drug treatment. This makes a comparison with the figures quoted for the optimal results less reliable. Recurrence of seizures is evident in approximately 25 % of the treated cases. Recurrences are primarily among patients previously reported as only improved by treatment. No major changes in the numbers of infants whose EEG and psychomotor development findings were improved after treatment occurred in this short observation time.

The significance of unknown re-

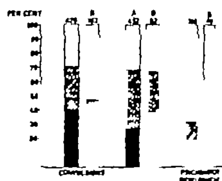


Fig. 1. Early results of treatment. Columns under A represent the early optimal results. Columns under B represent the results within one month after discontinuation of therapy. The solid area of each column indicates the percentual number normalized; the cross-hatched area the number normalized or improved, the hatched area the number improved. The figures above each column indicate the number of cases.

known etiology is illustrated in Table 2 and Fig. 2. The early optimal results are somewhat better in the former group when the cases are compared in which the convulsions have completely disappeared or the EEG has returned to normal. Concerning psychomotor development, the

TABLE 1. *Early results of treatment*

	Early optimal results		Results within one month after discontinuation of therapy	
	No. of cases	Per cent	No. of cases	Per cent
Convulsions disappeared	168	40.0	63	[illegible]
Convulsions disappeared or improved	[illegible]	8.3	—	—
Convulsions improved	107	[illegible]	15	[illegible]
Convulsions not improved	124	[illegible]	56	[illegible]
EEG normalised	119	27.4	14	[illegible]
EEG normalised or improved	22	5.1	—	—
EEG improved	144	[illegible]	10	[illegible]
EEG not improved	137	31.7	17	[illegible]
Psychomotor development normalised or unimpaired	29	9.7	4	[illegible]
Psychomotor development improved	66	22.0	13	[illegible]
Psychomotor development not improved	205	68.3	23	[illegible]

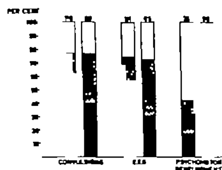


Fig. 2. Early optimal results in relations t unknown or "known etiology Columns under A represent cases in which the etiology has been unknown and columns under B those in which the etiology has been known. Symbols as in Fig 1

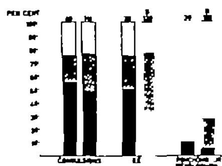


Fig. 3. Influence of lag of treatment. Limit one month. Columns under A represent cases in which treatment has been started within one month. Columns under B those in which treatment was started later Symbols as in Fig. 1.

results similarly seem to be better in the group of "unknown" etiology Psychomotor development had improved or returned to normal in approximately 40 % of these patients. However these figures refer only to the early optimal results and are not representative of the final state of the intellect.

The importance of early diagnosis and prompt initiation of treatment is illustrated in Tables 3 and 4 and Fig 3 and 4 With one month as a time limit the groups to be compared are of unequal size and this makes the comparison less reliable In general proportionally more cases are free from convulsions and more cases have a normal EEG in the group treated early Perhaps more important is the fact that

TABLE 2. *Results in relation to "unknown" or "known" etiology*

	Early optimal results in cases of un known etiology		Early optimal results in cases of "known etiology	
	No. of cases	Per cent	No. of cases	Per cent
Convulsions disappeared	64	33.2	36	40.0
Convulsions disappeared or improved	6	3..	5	5 6
Convulsions improved	10	16.4	28	31 1
Convulsions not improved	27	23.2	1	23.3
EEG normalized	41	36.9	40	20.0
EEG normalized or improved	3	4.3	2	4 6
EEG improved	37	33 3	24	36.9
EEG not improved	28	25.3	16	27 7
Psychomotor development normalized or unimpaired	13	17 6	4	6.9
Psychomotor development improved	18	24.3	12	20.
Psychomotor development not improved	43	59.1	4..	2.4

in the group treated within one month after onset of symptoms fourteen of thirty nine cases have normal psychomotor development compared to only seven of one hundred and thirty three (5.3 %) cases in the group whose duration of symptoms exceeded one month before the initiation of treatment These figures again give only the early optimal results of treatment

Groups of more comparable sizes are obtained using three months as a time limit Again better results are obtained with short treatment lapse Fifteen to twenty per cent more of the cases treated early are either free from convulsions or have a normal EEG when compared to the cases treated late Concerning psychomotor development 23% of the group treated early had developed normally as compared to 5% of the group treated more than three months after the onset of seizures. When the total number of either normal or improved cases is compared the difference is also clear but somewhat smaller A comparison between cases

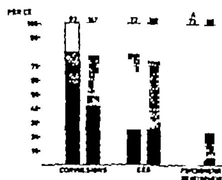


Fig 4 Influence of lag of treatment. Limit three months. Columns under A represent cases in which treatment was started within three months. Columns under B in which treatment was started later Symbol as in Fig 1

treated within either one or three months after the onset of seizures gives a greater percentage with normal psychomotor development in the former group, whereas no significant difference can be demonstrated regarding seizures or EEG

Discussion

From these results certain general trends are evident as to which variables influence the effectiveness of hormonal treatment of

TABLE 3 *Influence of lag of treatment on early optimal results Limit one month*

	Therapy started within one month		Treatment started later than one month	
	No of cases	Per cent	No. of cases	Per cent
Convulsions disappeared	22	55.0	59	44.4
Convulsions disappeared or improved	—	—	7	5.9
Convulsions improved	8	20.0	32	22.7
Convulsions not improved	10	25.0	33	23.6
EEG normalized	18	50.0	41	31.1
EEG normalized or improved	—	—	6	4.5
EEG improved	8	25.0	5	40.3
EEG not improved	8	25.0	30	22
Psychomotor development normalized or unimpaired	14	35.9	7	[illegible]
Psychomotor development improved	1	2.6	29	[illegible]
Psychomotor development not improved	24	61.5	97	[illegible]

TABLE 4. *Early optimal results in relations to lag of treatment. Limit three months*

	Cases treated within three months		Cases treated later than three months	
	No. of cases	Per cent	No. of cases	Per cent
Convulsions disappeared	53	57.0	61	41.5
Convulsions disappeared or improved	3	3.3	10	6.8
Convulsions improved	18	19.4	42	28.6
Convulsions not improved	19	20.4	34	23.1
EEG normalized	30	41.1	26	25.5
EEG normalized or improved	3	4.1	9	8.8
EEG improved	24	32.9	40	39.2
EEG not improved	16	21.9	27	26.5
Psychomotor development normalized or unimpaired	17	23.3	5	5.0
Psychomotor development improved	11	15.1	18	18.0
Psychomotor development not improved	45	61.6	77	77.0

infantile spasms. If only the early optimal results are considered, an unknown etiology and a short treatment lapse are favorable circumstances. Recurrence of seizures after cessation of therapy is fairly common.

Certain critical remarks are necessary in evaluating these results. The heterogeneity of the material has already been pointed out, for the selection is based on a type of epilepsy and not on a disease entity. This fact introduces the possibility of great variations in one series of cases from another. Also the proportion of cases with or without "known" etiology varies with the thoroughness of the diagnostic work up and with different opinions as to what constitutes a known cause of the brain disorder. A progressive disease such as demyelinating leucodystrophy is rare but of definite etiology. On the other hand neonatal asphyxia or hyperbilirubinemia is an acceptable cause of brain damage but does not by itself explain the usual progressive course of untreated infantile spasms with increasing mental deterioration. Additional mechanisms have been sought to explain how the initial brain damage may initiate a chain reaction but it is not clear if the fits in themselves may contribute to further brain damage or if other unknown factors (e.g. an autoimmune process) are involved.

It is possible that cases of unknown metabolic disturbances affecting the brain are hidden in the idiopathic group. In these cases ACTH may give better results than when structural changes have occurred in the brain. It has been mentioned that more cases in the group of "known" etiology have additional signs of brain damage such as cerebral palsy and this may indicate that irreversible changes are present to a greater extent in this group.

A major handicap in evaluating the different reports has been the varying degrees of accuracy with which the child's psychomotor development has been judged. This assessment has unfortunately often been limited to superficial impressions such as "better contact with surroundings", "appears more lively", "more

active" etc. Only rarely has a thorough longitudinal psychomotor evaluation with accepted psychological developmental tests been used. Relatively few investigators have followed up their material for any length of time and not all of them have used accepted psychometric testing in the final evaluation. The problem of long term results with a more detailed review of previous findings will be reported in the Swedish cooperative study

The better effect especially on psychomotor development, with a shorter lapse of time before the initiation of treatment is not surprising However the age factor may play a role here for those cases coming earlier for treatment are possibly also somewhat younger than those coming later This factor has not been systematically analyzed in the reviewed literature but the age factor in the Swedish study has not been of major importance Another possible source of error may be that those infants whose development was normal before the onset of seizures were brought sooner to a physician and were sooner diagnosed. It is likely that this group is somewhat better off for any therapeutic trial.

The choice between ACTH and corticosteroids, dosages and duration of treatment are factors which the present report does not analyze Large doses of ACTH for three to four weeks have been generally preferred but no controlled study has been made to compare the effect of ACTH versus corticosteroids. It is common to increase the dose to very high levels in patients who do not respond to small or moderate doses This makes it almost impossible to study the effect of treatment of varying dosages.

Some authorities claim that the results with ACTH or corticosteroids are only symptomatic and analogous to the anticonvulsive effect of phenobarbital and dilantin. This statement is somewhat contradicted by the fact that the effect of hormone treatment persists in most cases after cessation of drug therapy Also the effect on the EEG is often dramatic which is not a common observation when conventional anticonvulsive drugs are used in epilepsy (the effect of tridione on petit mal is an exception)

Summary

A review of published cases of infantile spasms and hypsarhythmia treated with ACTH and corticosteroids has been made and combined with preliminary data from a Swedish cooperative study Four hundred and seventy cases have been analyzed The effect of treatment on the incidence of seizures, the EEG and psychomotor development has been compared in different groups. Cases of unknown etiology and cases which are treated shortly after the onset of seizures show better results. The incidence of early recurrences has been estimated. The more favourable results concerning psychomotor development in the groups studied may be of great importance but final evaluation must await more thorough longitudinal studies with better psychometric methods and longer follow-up

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Department of Pediatrics
Karolinska Sjukhuset
Stockholm
Sweden

active" etc Only rarely has a thorough longitudinal psychomotor evaluation with accepted psychological developmental tests been used. Relatively few investigators have followed up their material for any length of time and not all of them have used accepted psychometric testing in the final evaluation The problem of long term results with a more detailed review of previous findings will be reported in the Swedish cooperative study

The better effect especially on psychomotor development with a shorter lapse of time before the initiation of treatment is not surprising However the age factor may play a role here for those cases coming earlier for treatment are possibly also somewhat younger than those coming later This factor has not been systematically analyzed in the reviewed literature but the age factor in the Swedish study has not been of major importance Another possible source of error may be that those infants whose development was normal before the onset of seizures were brought sooner to a physician and were sooner diagnosed. It is likely that this group is somewhat better off for any therapeutic trial

The choice between ACTH and corticosteroids dosages and duration of treatment are factors which the present report does not analyze Large doses of ACTH for three to four weeks have been generally preferred, but no controlled study has been made to compare the effect of ACTH versus corticosteroids. It is common to increase the dose to very high levels in patients who do not respond to small or moderate doses. This makes it almost impossible to study the effect of treatment of varying dosages.

Some authorities claim that the results with ACTH or corticosteroids are only symptomatic and analogous to the anticonvulsive effect of phenobarbital and dilantin This statement is somewhat contradicted by the fact that the effect of hormone treatment persists in most cases after cessation of drug therapy Also the effect on the EEG is often dramatic which is not a common observation when conventional anticonvulsive drugs are used in epilepsy (the effect of tridione on petit mal is an exception)

Summary

A review of published cases of infantile spasms and hypsarhythmia treated with ACTH and corticosteroids has been made and combined with preliminary data from a Swedish cooperative study Four hundred and seventy cases have been analyzed. The effect of treatment on the incidence of seizures, the EEG and psychomotor development has been compared in different groups. Cases of unknown etiology and cases which are treated shortly after the onset of seizures show better results. The incidence of early recurrences has been estimated. The more favourable results concerning psychomotor development in the groups studied may be of great importance but final evaluation must await more thorough longitudinal studies with better psychometric methods and longer follow up.

most frequent cause is intestinal obstruction. This occurs particularly frequently in premature infants. The diagnosis must be considered in every case with symptoms suggestive of intestinal obstruction in newly born infants. The diagnosis can be confirmed by radiography of the abdomen with horizontal rays when the intraperitoneal air will become clearly visible particularly with the child in the hanging position.

Case 1 Birth weight 3100 g At laparotomy on the fifth day of life perforation of the caecum was found. This was later shown to be due to congenital megacolon. The patient is alive at the age of six years.

Case 2. Birth weight 1240 g. During the second day of life perforation of the distal part of the ileum occurred where pronounced muscular hypoplasia in the intestinal wall had caused functional intestinal obstruction. The patient died immediately after laparotomy

Case 3: Birth weight 2100 g On the third day of life the patient developed pneumoperitoneum but no perforation could be found on laparotomy The patient made a good recovery after operation and the intestinal function is good.

As soon as the diagnosis of pneumoperitoneum is established, this is an absolute indication for laparotomy and further diagnostic procedures, particularly the administration of radio-opaque substances, are contraindicated.

DISCUSSION

K Mauritzen Nine cases of intestinal perforation in infants admitted to the Paediatric Department Rigshospitalet are mentioned. Four infants submitted to operation survived, two died after operation and three were not submitted to operation (moribund on admission). Four of the infants were premature Out of eight cases, pneumoperitoneum was demonstrated pre-operatively in three cases but was not seen in the X-ray photographs in three cases in which perforation was found at operation and in two cases not submitted to operation where perforation was found at autopsy In one case, no pre-operative radiographs were taken. After perforation demonstrated at operation, pneumoperitoneum was found retrospectively in two further cases on review of the pre-operative radiographs. The aetiology was Hirschsprung' disease in two cases, volvulus in two cases and meconium ileus in one case The perforation was found in the sigmoid colon (4 cases) the transverse colon (1 case) the ascending colon (1 case), the caecum (1 case) and the small intestine (cases) In six cases, the perforation was considered to be spontaneous, in three cases, perforation may have been caused by a rectal sound and in one case an enema may have contributed to perforation. Radiography after radio-opaque enema was undertaken in a total of four cases. Radio-opaque enema should not be administered in cases of pneumoperitoneum where the indications for operation are obvious. In doubtful cases, radio-opaque enema may be carried out with careful technique and low pressure with an aqueous solution of the radio-opaque medium.

Meeting March 21 and 22, 1964

S Vestermark: Endocardial Fibroelastosis

A series comprising eight boys and nine girls with primary endocardial fibroelastosis was presented. In 50% of the cases, the symptoms started within the first three months of life and in 80% within the first six months. The clinical picture was non specific. The diagnosis was established by means of the typical angiocardiographic picture and was confirmed in ten cases by autopsy or biopsy Typical ECG findings with ventricular hypertrophy and negative T waves in lead V 5–6 were found in ten cases. No relationship could be demonstrated

between the time of commencement of the disease and the course of the disease. Eight of the patients had not received any treatment and seven of them died. Nine of the patients had received digitalis and two of these had died from cardiac failure, two died from accidents while five are alive and symptomfree.

Sv. Brandt, K. Hauge Kristensen and P. Plum. Is the Incidence of Cerebral Paresis Decreasing?

In the Clinics for Cerebral Palsy in Rigshospitalet and the Orthopaedic Hospital during the years 193 -1939 a total of 43 new cases in children from 0–13 years were diagnosed. This figure had fallen to 355 for the period 1900–190. The total decrease of 18% is statistically significant. Further analysis of the material revealed a percentually even greater reduction (viz. from 100 to 58) in the diagnostic group "spastic tetraplegias and children with athetosis" (in the two periods concerned, 161 and 94 new cases respectively had been diagnosed). Similarly a statistically significant decrease from 246 (100%) to 176 (9 %) was found in the group of children who were under the age of three years at the time of reference to the clinics. On the other hand no statistically significant decrease was found in the age groups -5 years and 6–13 years, respectively, nor in the diagnostic groups "spastic hemiplegias", "spastic di and paraplegias" and "ataxias".

The figures from the Cerebral Palsy Clinic at the Orthopaedic Hospital also showed a decrease in the number of severe and moderately severe cases and a corresponding increase in the number of slighter cases. These figures were however too limited to be considered statistically significant. A similar decrease in the number of cases of cerebral paresis referred to hospital is reported from Bristol in England by Grace Woods.

On the basis of these findings, the authors cannot yet decide whether this decrease reflects the first gratifying results of better prophylaxis. It is definite, however, that the decrease in the number of cases of kernicterus must at any rate be contributory. Another possibility is that investigation of small children with severe forms of cerebral palsy (tetraplegias and athetosis) is primarily undertaken at other clinics, possibly at the Clinic for Mentally Subnormal Children in Copenhagen to a greater extent than previously. Finally it must also be supposed that the decrease in the number of newly diagnosed cases of tetraplegia and athetosis in small children may be connected with expansion of the diagnostic work elsewhere in Denmark.

DISCUSSION

J. Flamand Christensen. With the object of investigating whether a corresponding alteration in the incidence of the various types as in the material from Copenhagen has occurred on the Island of Funen, I have reviewed the series from the Out-Patient Clinic for Cerebral Palsy in the Municipal and County Hospital in Odense until 1962. A total of 149 children were involved and, on subdivision of these into the same four groups as in the material presented by Brandt *et al.*, no alteration in the distribution in the course of the years was observed. The figures in the individual groups per annum are however so small that evaluation is unreliable. On investigation of the years of birth of the children in question, it was demonstrated that between 7 and 20 children with cerebral palsy are born in Funen annually, the average being 13. As approximately 8200 children are born annually on Funen and the surrounding islands, this gives an incidence of 1.6‰, which corresponds to the incidence found by Erik Hansen for Denmark as a whole. — *J. Lenstrup.* Information concerning a possible decrease in the number of cases of cerebral damage with spastic paresis in children is of the greatest interest for the authorities of the Care of the Mentally Subnormal who are at present engaged in planning and extension. The number of hospital beds is being increased but the

greatest interest is being focussed on centres for treatment of children living in their own homes in recognition of the great significance both for children and their parents f close mutual contact, particularly during the child first years. For teaching and physiotherapy of not only children with slight cerebral damage and also severely spastic children, nursery schools, schools, vocational schools and day homes are being planned. The authorities of the Care of the Mentally Subnormal are eager t participat in in estigations which can illustrate the need for treatment centres in future The Children Department of the Care of the Mentally Subnormal for Copenhagen had connection with 143 children with the diagnosis of cerebral palsy in varying degrees since April 1960 These cases are reported. Further a detailed account is given of the number of cases referred during the periods 1956–1958 and 1959–1961 The majority of children were referred within the first eight years of lif as a rule in connection with educational requirements. During the first period, 41 children were referred and, in the second period, 62 children. This increase is probably ttributable to the inauguration of the Advisory Clinic f the Children Department and to the increasing confidence of the population in the services offered by the Care of the Mentally Subnormal. In the communication by Brandt *et al.*, the possibility is envisaged that the decreasing figures in the clinics concerned were possibly connected with an increasing number of cases which by pass the clinics and are referred directly to the Children Department of the Care of the Mentally Subnormal. This is pparently not the case *Review of the case histories of the 103* children who were referred in the periods mentioned reveals that at least 93 of these patients are known in the clinics concerned. — *Erik Hansen* A genuine decrease in the incidence of cerebral paresis is scarcely concerned but rather a displacement of the patient material so that other departments .g. paediatric departments in the provinces and department under the Care of the Mentally Subnormal now investigate and follow-up patients with cerebral palsy to a greater extent If a genuine figure for the incidence is to be obtained, the investigation instituted by the Association for Spastic Children in 1935 must be brought up to dat Caution should be exercised in ttributing the presumed decrease in the incidence of cerebral paresis to better obstetric technique A considerable improvement ha occurred in the treatment of newborn infants with cerebral symptoms so that in this manner the lives of many infants have been saved and these may be potential cerebral palsy patients.

Gudrun Salem. Persist nt Ductus Arteriosus with Pulmonary Hypertension in Children under Two Years

Twenty children under the age of two years were submitted to operation for persistent ductus arteriosus with pulmonary hypertension. These children should be operated as soon as the diagnosis is established partly on account f the immediate risk which the disease implies and partly because pulmonary hypertension frequently persists after operation if this is postponed until a later age In contrast to this, investi gations undertaken in this department have shown that the pulmonary pressure becomes normal (less than 40 mm Hg) if the duct is closed in infancy The *0 children were assessed in view of the diagnosis. In 14 stethoscopic examination aroused suspicion as, in these cases, the murmur was of maximal intensity in the second left intercostal space but a typical continuous *murmur was found in four cases only ECG* may be of assistance in that signs of right sided ventricular hypertrophy are evidence against the presence of an isolated persistent ductus arteriosus. In 15 out of 19 children straight radiography raised suspicion of the diagnosis in that increased vascular markings in the lung fields were present in 14 while in ten, the pulmonary arch was prominent

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Department of Pediatrics
University Hospital
Oslo
Norway

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CASE REPORT

Spontaneous Hypoglycaemia with Convulsions and Deficient Adrenaline Reaction

A Case Occurring in One of Uniovular Twins

by ANNA MADSEN

Department of Paediatrics, Gentofte Hospital (Head: P. W. Braestrup, M.D.) Hellerup, Copenhagen, Denmark

In the Paediatric Department of Gentofte Hospital we have had the opportunity of observing a child, one of uniovular twins, who had spontaneous hypoglycaemia and an insufficient adrenal reaction to insulin-induced hypoglycaemia. This defect was first described by Broberger, Jungner & Zetterström who published 3 cases in 1959 [1], and 4 further cases in 1961 [2]. In 1963 Brunjes and his colleagues [3] published report of 8 cases. From Denmark Kildeberg [9] published in 1963 the report of a case of spontaneous hypoglycaemia in which the absence of symptoms of adrenaline secretion, such as sweating and tachycardia, could point to the same pathogenesis.

The first case of hypoglycaemia—in a 2 year-old boy—to be published from Denmark was that of Lenstrup in 1926 [10]. The aetiology of this case was not established, but the condition was improved by frequent high-carbohydrate meals. Since 1954 when McQuarrie [14] published 40 cases of spontaneous hypoglycaemia in children, of which only 15 could be classified according to the types known at that time, this disorder has been known as McQuarrie's syndrome or spontaneous idiopathic hypoglycaemia in children.

In 1956 Cochrane and his colleagues [5] demonstrated that the amino acid leucine, which contains almost all the albumin compounds found in the normal diet, can cause hypoglycaemia in sensitive subjects. Since that time many cases of leucine sensitivity have been described, in Denmark by, among others, Lundbæk [11], who stated that one third to one half of the children with so-called idiopathic spontaneous hypoglycaemia are sensitive to leucine.

Case History

The patient is a boy, born Sept. 7, 1959, a uniovular twin. The pregnancy went to term, birth weight 2500 g. No member of the family has had convulsions or abnormalities of carbohydrate metabolism. The patient's twin brother is alive and completely healthy.

At the age of 9 months admitted for the

first time with alimentary anaemia, no con vulsions.

At the age of ½ years admitted for the second time because of convulsions. He had frequently suffered from diarrhoea—including during the _ days prior to admission—and also from vomiting On the day of admission there were universal clonic convulsions with loss of consciousness at 9.30 a.m., 11.30 a.m and 1.30 p.m.

On admission at 2 p.m. no convulsions. Blood sugar (Hagedorn-Jensen) 30 mg/100 ml, spinal sugar 2 mg/100 ml. Cerebrospinal fluid otherwise normal. Serum bicarbonate 12 mEq/l. Urine: cetone present no sugar Temperature normal. Ophthalmological examination. convergent strabismus, normal fundus. X ray of the skull in 4 projections: normal. EEG: slightly abnormal. H was treated with glucose and bicarbonate in travenously but did not complet ly regain consciousness until the following morning Was well during the remainder of his ad mission It was concluded that this was a case of gastro-enteritis, with secondary hypoglycaemia with convulsions. No therapy was prescribed after discharge

Admitted for the third time 3 months la ter in July 1962. During the intervening period he had been well. On the day of ad mission at 10 a.m, 2 hours after his break he suddenly f ll and vomited. The vomiting continued until after his admission t 11.30 a.m. He was apathetic, flushed and his eyes were turned downwards. There was *the strabismus which had been observed* previously quivering of the right corner of the mouth, and possibly also of the left, and bilateral extensor plantar responses. The temperature was normal. Blood sugar 42 mg/100 ml. Serum bicarbonat 12 mEq/l. The urine contained acetone but no sugar EEG which had been normal 8 days before admission, was now found to be moderately abnormal over the right occipito-parieto-temporal region, significant for epilepsy The patient was treated with sweetened fluids by mouth, and he returned to normal in the course of the afternoon. The remainder of the usual laboratory investigations revealed nothing abnormal, except that the excretion of 17 ketosteroids was found to be t the upper limit of normal on first investigation, but not definitely increased on second investigation. Glucose tolerance test was normal on the first occasion; the second time there was a delay in the rise in blood sugar, which could *suggest gastric retention*, but X ray of the stomach revealed that the emptying tim was normal. Twelve days after admission the EEG was not definitely abnormal.

After the extended blood sugar investigations (see later) the child was treated with ephedrine 5 mg twice daily from Aug 29, 196_, until May 28, 1963. During this period he had no attacks and was completely w ll; in particular there was no evidence of excit tion. After withdrawal of the therapy the child perhaps required slightly more snacks between meals. Aug 9 1963, another universal convulsion, this time connected with a pyrexia of unknown origin. From Aug 10, 1963, afebrile but as he was still troublesome and difficult he was admitted for the fourth time Aug 13, 1963. Examination revealed nothing abnormal. ESR 4 mm Hb 103%. *Aug 23 1963*, new insulin tolerance test. EEG on several occasions during admission and as out patient normal or slightly abnormal; in general normal without provocation, but 3–4 cps activity and spikes over the occipito-parietal region during photostimulation and sleep. Renewed treatment with ephedrine at the same dosage (*5 mg 2*) continued since *Aug* 4 1963. He has been followed up as an out patient the last occasion being June 9, 1964. He has been completely well, no excitation. H has developed in exactly the same way as his twin brother

Special Investigations

Fig. 1–6 show the results of the special investigations which have been carried out

The fasting blood sugar (Fig 1) shows an obvious fall to hypoglycaemic levels after 15 hours, which is possibly pathological. According to Wilkins [17] this excludes in



Fig. 1. Blood sugar during 4 hours fast. Abscissa: time in hours. Ordinate: blood sugar in mg per 100 ml.

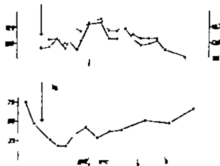


Fig. 3. Insulin tolerance test, using 0.4 i.u. crystalline insulin per kg intravenously. Arrow indicates time of injection ( ) Abscissa: time in hours. Ordinate on left: systolic blood pressure. Ordinate on right: pulse. O——O systolic blood pressure; —— pulse (b) Abscissa: time in hours. Ordinate: blood sugar in mg per 100 ml. K convulsion; C cyanosis; O vomiting

adenoma. There were no symptoms of hypoglycaemia during the test.

Leucine tolerance test (Fig. 2) showed some fall in the blood sugar—such is often seen in insulin sensitivity—but not such a pronounced fall as that seen in typical leucine-provoked hypoglycaemia. The same result was obtained after both oral and intravenous administration.

Insulin tolerance test (Fig. 3) using 0.4 i.u. per kg body weight intravenously provoked a severe and long-lasting fall in blood sugar. There were convulsions, cyanosis and vomiting. This result emphasizes the danger of this investigation and the desirability of—as was the case during the investigation under discussion—having access to an open vein for use for a rapid intravenous glucose injection, when this is necessary.

On repetition of the insulin tolerance test (Fig. 4), principally the same result was obtained, although after the smaller dose of insulin the reaction was less marked. During the first investigation the pulse and blood

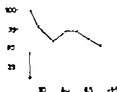


Fig. 2. Leucine tolerance test. Abscissa: time in minutes. Ordinate: Blood sugar in mg per 100 ml. At the arrow 75 mg/kg l-leucine was given intravenously.

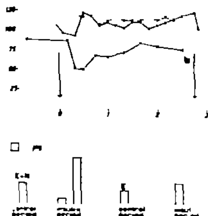


Fig. 4–5

Fig. 4. Insulin tolerance test, using 0.1 i.u. crystalline insulin per kg intravenously. Abscissa: time in hours. Ordinate: blood sugar in mg per 100 ml, systolic blood pressure and pulse. —— Blood sugar; —— systolic blood pressure; O——O pulse. Arrow (at time 0) marks injection of insulin; at arrow b glucose was given intravenously.

Fig. 5. Urinary excretion of catechol amines during 2 insulin tolerance tests. Insulin tolerance 1: 0.4 i.u./kg. Insulin tolerance 2: 0.1 i.u./kg. \ noradrenaline; E = adrenaline

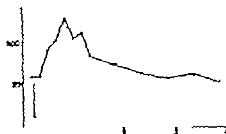


Fig. 6. Adrenaline test. Abscissa: time in hours. Ordinate: blood sugar in mg per 100 ml. At arrow 0.2 ml adrenaline given intramuscularly.

pressure were to some extent affected during the period of hypoglycaemia.

Fig. 5 illustrates the adrenaline/noradrenaline excretion in the urine during insulin hypoglycaemia. The total excretion during the first 3 hours after the administration of the insulin is compared with a 3 hour control period. The normal 5–10 times increase in excretion as compared with the control period did not occur.

By following the blood sugar level after an injection of adrenaline (Fig. 6) it was shown that the child reacted to the adrenaline by a normal rise in blood sugar.

Discussion

During the first insulin tolerance test analysis of the urinary excretion of catechol amines revealed an increase in the total adrenaline to 2½ times that of the control period. The main part of this increase comprised noradrenaline whilst normally 70% of the increase consists of adrenaline. The amounts excreted during the control period were too small for fractionation but the results were however considered to be so significant of an absence of the adrenaline reaction that treatment with ephedrine was started, and the boy became well on this treatment.

As there is a tendency for spontaneous hypoglycaemia in children to remit at the age of about 3½ to 4 years (although in one case the symptoms of the deficiency of adrenaline lasted until the age of 9 years) we considered it reasonable to attempt to withdraw the ephedrine when the patient reached the age of 3 years 9 months. Two and a half months later he suffered from another universal convulsion—there is, however, a possibility that this was provoked by fever, which had not been the case on previous occasions. The insulin experiment was repeated, and this time it was possible to fractionate the total adrenaline in both samples of the urine (see Fig. 5). The results were definitely pathological, as the excretion of adrenaline during the hypoglycaemia was not even doubled. *That is, the patient suffers from Zetterström's type of spontaneous hypoglycaemia.*

After this experiment the ephedrine therapy was recommenced and it seemed still to be effective. It is our intention to continue this treatment for a long time, presumably several years.

The true cause of the hypoglycaemia in this group of patients has still not been established. There have been discussions as to whether it is centrally or peripherally determined—that is, whether the defect is localized in the hypothalamus or the adrenal medulla or possibly in extramedullary chromaffin tissue. Both Broberger & Zetterström [2] and Brunjes et al. [3] seem most inclined to consider that the adrenal insufficiency is of central origin, as many of these children show other evidence of cerebral damage. Thus Broberger & Zetterström's material included one child in whom the defect first developed after a serous meningitis.

The findings from our patient give no

support to either of the theories There was no birth trauma The birth weight must in consideration of the fact that he was a twin be considered to have been normal. The EEG findings cannot be considered conclusive as the slight changes observed could well have been the sequelae of the attacks from which the patient had suffered, especially as the first EEG taken on the day following the first convulsion, showed the small changes described, whilst control EEG 3 months later was completely normal.

The fact that during the first insulin tolerance test there was some clinical adrenaline effect during the first hour of the insulin-induced hypoglycaemia would suggest that in all events the adrenal medulla was functioning, as in cancer patients who have undergone adrenalectomy the adrenaline response is not observed until the third hour of the hypoglycaemia (third hour response) and presumably originates in the extramedullary chromaffin tissue [16]

When one has no explanation of a defect of this nature the possibility that it is genetically determined must be considered and in the present case thi possibility can be elucidated very convincingly The mother st tes that there was on placenta and according to the attending bstetrician the babies were uniovular They re the image of one another both having red hair and blue irides As an extra precaution blood group investigations have been carried out at Statens Seruminstitut Copenhagen, and have revealed that both children have the blood groups A Rh+ CDe/C e MNS P[pos] Le (a) k/k Fy (a-)

According to the Institute of Genetics Copenhagen, the probability that with these findings the twin are uniovular is about 99.5 .

This provides very strong evidence against the possibility that the defect is genetically determined.

Within 2 years Broberger & Zetterström examined 11 children with hypoglycaemia 5 of whom were shown to have adrenaline defect This indicates the necessity of carrying out special investigations when the cause of the hypoglycaemia is not obvious. The diagnosis and therapy are important as the hypoglycaemic attacks can lead to irreparable cerebral damage which seems to be most severe when the child develops attacks at an early age and when the attacks are frequent and severe

Summary

A report is given of a case of spontaneous hypoglycaemia associated with a deficient adrenalin reaction to the hypoglycaemia The patient is a uniovula twin with a healthy twin brother nd it is therefore unlikely that the disorder is genetically determined. The importance of early diagnosis and treatment is emphasized.

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Hasselvej 22
Virum
Denmark

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CASE REPORT

Cor Triloculare Biatriatum Associated with Klippel Feil Syndrome

by KJELL J TVETER and TROND KLUGE

From the Oslo University Institute of Pathologic Anatomy, Rikshospitalet Oslo, Norway
(Head Professor O Torgersen, M.D)

Introduction

Advances in therapy demand that every case of congenital heart disease be subject to a careful investigation, in order that an operative correction may be performed when indicated, and to avoid surgical intervention when this is of no benefit

Most congenital cardiac anomalies can today be revealed when all modern diagnostic procedures are available but there will always remain a small group of defects where an accurate diagnosis is difficult The present case of cor triloculare biatriatum is characterized by a defect which was mis-interpreted by all departments concerned, and even—in the first hand—by the pathologist

Case Report

The patient B H., was a girl born on September 20 1963, 3 weeks before expected delivery The pregnancy was normal. Apart from an inguinal hernia, the patient postnatal condition was satisfactory with normal gain in weight At 3 months of age she presented the first signs of cardiovascular disease expressed by attacks of dyspnoea and cyanosis. Such attacks, lasting from a few minutes to 3 hours, increased in frequency On January 20 1964, she was admitted to a provincial hospital because of purulent rhinitis combined with the above-mentioned attacks. She was treated with sufficient amounts of antibiotics, but her dyspnoea did not diminish.

On February 7 she was transferred to Rikshospitalet Oslo. On admission she was cyanotic with a respiratory rate of 80/min. Pulse regular 138/min. No finger or nail-affections were observed, neither were there any abnormal pulmonary sounds. The cardiac apex beat could not be felt. The heart action was regular but auscultation revealed a systolic murmur of III degree, with a maximum in the 3rd left I.c space The 2nd pulmonary sound was accentuated. The liver was palpable 3 cm below the costal arch.

The *ECG examination* demonstrated a moderate right hypertrophy and digitalis-pattern in the standard leads (Fig. 1), and negative deflection of the QRS-complexes in the precordial leads V 1 V 4 *X-ray-examination* (Fig 2) revealed marked dilatation of the right ventricle and possibly of the right auricle In addition, numerous anomalies were observed in the lower cervical and upper thoracic spine with fused and cleft vertebrae and fusion of ribs, consistent with a Klippel Feil syndrome *Cardiac catheterization* was performed on February 14 On attempt to pass the catheter from the right auricle into the ventricle a 2.1 A V block occurred, the beat rate falling from

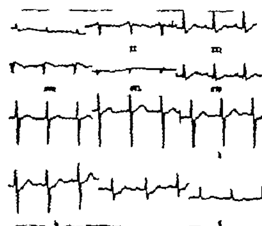


Fig. 1 ECG registration. Standard leads show right hypertrophy and digitali pattern. Negative deflection of the QRS-complex in precordial leads 1–4.

155 to 6" Angiocardiography therefore was performed with the catheter in the right auricle. Determination of the oxygen saturation showed the following pattern: Superior vena cava: 9 %. Right auricle (middle position) 10 %. Inferior vena cava: 30 %. Left superior vena cava 4 %. Left auricle 98 %. Due to the position of the catheter ventricular blood samples were not available. The rise in oxygen saturation from the superior vena cava to the right auricle (1 %) and the *fall* from the *inferior* vena cava to the auricle (10 %) are both within the normal range. The only conclusion to be drawn was that a shunt at atrial level was most improbable. The angiocardiography (Fig. 3) demonstrated a rapid filling of the aorta, which was considered over riding. Within a short interval, filling of the pulmonary artery was obtained, this being slightly smaller in size than the aorta.

The conclusion from the clinical picture and the above investigations was a congenital heart defect consistent with a tetralogy of Fallot. On March 3, the patient was subject to surgical correction with a transventricular valvulotomy and infundibulotomy. The patient died some hours later



Fig. 2. Chest X-ray, anterior view. Marked cardial dilation, mainly of the right portion. Besides, anomalies of the spine and ribs are noted, consistent with Klippel-Feil syndrome (see arrows).



Fig. 3. Angiocardiography anterior view with the catheter placed in the right auricle (*RA*). Note simultaneous filling of the aortic arch (*AA*) and the pulmonary artery (*PA*), the latter being of almost normal caliber. *CVW* wall of common ventricle.



Fig. 4. Heart right lateral view [illegible] he wall of he common ventricle (*CV*) [illegible] opening (*O*) of the channel leading into the rudimentary outlet chamber.

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Institute of Pathologic Anatomy
Oslo University
Rikshospitalet
Oslo
Norway

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CASE REPORT

Acute Cerebellar Ataxia in Children Associated with Coxsackie Viruses Group B

by REBEKKA BERG and HUGO JELKE

From the Institute of Virology, University of Uppsala, and the Children's Hospital, Gävle, Sweden

Acute cerebellar ataxia is a syndrome which has attracted special attention on account of the prevalence in infancy and early childhood, the often alarming onset and the uncertainty concerning the etiology. The condition can occur throughout childhood, the age of preference being one to four years. In about half of the patients signs of infectious disease are noticed from several weeks to immediately before or even during th condition. Recovery usually takes place from about one week up to half a year after onset f illness [10 13, 29]

Conceptions of what ought to be included under the term acute cerebellar ataxia differ widely The following criteria fit the strictest requirements for a diagnosis—sudden onset of symmetrical cerebellar signs in a previously healthy child without prodromata, fever nuchal rigidity abnormalities of cerebrospinal fluid or possibility of intoxication [7]

Most of the cases published show cerebellar and cerebral sympt ms in different combinations [8 12, 16, 28]

It is generally accepted that acute cerebellar ataxia has a favourable prognosis. is true *quoad vitam*. According to Brorson [4], however not necessarily *quoad restitutionem* the long term prognosis was found to differ considerably

The etiology of acute cerebellar ataxia seems to be heterogeneous and not fully established. Acute cerebellar ataxia has been encountered in association with polio [2, 20 22, 30], upper respiratory disease [11 17], mononucleosis [14] varicella [–5 *7], measles [*6], influenza [3], mumps, rubella, variola scarlet fever whooping cough, diphtheria and after intoxication [15] An anaphylactic reaction in the cerebellum to different agents has also been suggested [6 0 23] It seems, however that a diagnosis has been properly established through laboratory evidence only in the cases of Arthuis *et al.* [1] (Polio type 1) Berglund *et l* [3] (Polio type 1) McAllister [19] (ECHO type 9) Curnen & Chamberlin [7] (Polio type 1) and Meyer [*1] (Coxsackie group A type 2).

To our knowledge the Coxsackie viru ses group B have not been established by laboratory evidence as etiological agents in acute cerebellar ataxia.

This report deals with the clinical and virological findings for tw patients with

acute cerebellar ataxia from which a coxsackie virus group B was isolated and a significant increase in antibody titer against the homologous strain was found during the illness.

Case Histories

Case 1

(J 648/63) A 19-month-old girl, Lena J., born April 10 196.., was admitted to the hospital November 25 1963, with the diag nosis acute cerebellar taxia. Birth and developmental history were normal, she walked at the age of ten months. Not polio-vaccinated She had been asymptomatic until one month before admission when she developed a limp of unknown etiology and lasting a few days in one leg Again she was asymptomatic until 4 days before entry when she vomited once. November 23 she stumbled and had a tendency to deviate. On getting out of bed on the morning f November 25 she showed marked unsteadi ness and could hardly stand on her feet. She improved slightly during the day

On admission her general condition was good. Her gait was broadbased and unsteady and she showed a tendency to fall. During movement she revealed a slight intention tremor Aside from the signs reported and a slightly positive Pandy's reaction, the exa mination of the patient—neurological, otological, ophthalmological and ordinary la boratory examination included—showed con ditions within normal limits.

Course The intention tremor disappeared two days later Even the ataxic gait subsided quickly and steadily Six days after the onset of illness she walked steadily and with normal balance.

The patient was discharged on December 9 asymptomatic with the exception of a remaining Fournier i.e she was ble to stand on one leg only when her eyes were open.

Case 2

(J 519/63) A nine-year-old boy Jan E., born May 7 1954, was admitted to the hosp tal September 8, 1963 under the diagnosis of acute encephalitis. In 1960 he had been hospitalized for two weeks with an acute tracheobronchitis. Morbilli was contracted in 1961 and ran an uneventful course Polio-vaccinated three times.

About August 25 1963 he suddenly developed fever up to 40°C without other symptoms. The fever subsided in 24 hours. He was asymptomatic until September 2, 1963 when he felt tired. From the next day he had continuous nausea and vomited repeatedly For the next three days he went to school as usual. September 6 he was sent home from school. The next day he got a headache and toward the evening fever up to 38.5°C. September 8, his gait was unsteady and he had difficulty in keeping his balance. The doctor who saw him admitted him to the hospital with the diagnosis suspected encephalitis.

On admission he appeared to be moderately ill. H was tired, pale, but totally conscious and aware of his surroundings. Temperature 37.3°C. Slight nuchal rigidity and positive Kernig Continually strong, jerky eye-movements were accentuated by at tempt at fixation. During examination of the masseter reflex, which was increased, flashing jerks in all muscles of the face were noticed. The finger-to-nose test revealed slightly intention tremor The heel-to-knee test was performed clumsily Romberg test positive. The patient was able to sit up and to stand up It was difficult, however for him to stand on his feet for a moment without support and he succeeded only if he did so with a wide base. This revealed a very coarse tremor in the muscles of his legs, simulating a cold shiver When he was able to take couple of steps he staggered to the side

A lumbar tap gave clear cerebro-spinal fluid under normal pressure (80 mm). The Pandy reaction was slightly positive and the Nonne reaction negative. Protein 80 mg%. The cell count showed 16 per mm³ 10 polymorphonuclears and 8 mononuclears. The sugar was 57 mg%; blood sugar was 133 mg%.

The EEG recorded a normal background

rhythm postcentrally of 8 /sec. Over the posterior quarters slower activity (4–7 c/sec) together with slow polycyclic potentials were found. In view of the age of the patient these were considered within normal limits. Aside from the pathological findings reported, neurological, otological and ordinary laboratory examination showed conditions within normal limits.

Course The first week the boy was subfebrile with temperatures up to 38.2°C. He had nausea and vomited several times daily especially when he moved his head. Later on he was afebrile Twelve days after onset pleocytosis increased to 84 cells per mm³ predominantly mononuclears, and later diminished t six.

On the third day the marked tremor of the eyes began t subside, after another three days it disappeared completely

In the lower extremities tremor persisted for ten days after onset when he attempted to get on his feet. His balance and gait improved. After a fortnight he walked steadily Romberg test was then negative but Fournier remained positive so that on discharge he still was able t stand on one leg only when his eyes were open.

Apart from this he was asymptomatic at this time.

After discharge, October 8, 1962, a mild change in emotional stability and an increased irritability were observed. H has been well subsequently

Virological Examination

Feces, throat swabs, cerebrospinal fluid, acut and convalescent sera were sent to the regional diagnostic laboratory The material for virus isolations was treated as described earlier [31] and inoculated in 0.. ml amounts in each of 2 cynomolgus kidney and 2 KB tissue culture tubes maintained in Eagle basal medium without serum. The cytopathic agents isolated were passaged and primarily typed against about 50 neutralizing unit of hyperimmune rabbit serum against the type strains of polio type 1 .., 3, ECHO type 6 and 9 and the Coxsackie viruses group B type 1–5. Af er the first typing, the isolated virus was titrated and retyped. About 100 TCD_{50} of the isolated strains and 50 neutralizing units of the type serums were used

The convalescent sera of the patients were screened in neutralization tests in dilution 1/20 against 100 ID_{50} of poliovirus type 1 2, 3, ECHO virus type 6 and 9 and the Coxsackie viruses group B type 1–5 The complete set f positive sera from each patient was then titrated in 2 fold dilutions against 100 ID_{50} of the virus types. Complement fixation test were performed against Polio type 1 2, 3 Herpes Simplex, Influenza A, B, Parotitis and Adeno viruses. In addition Bunnell' reaction was performed.

From the feces and throat swab taken on the 6th day after onset of illness in Case 1 two virus strains were isolated. The two strains were typed as Coxsackie virus group B type 4. The complet set of serum samples from the patient showed insignificant decreasing titer against Coxsackie virus group B type 3 but more than fourfold increase in antibodies against type 4 and the homologous strain.

From the feces from the 5th day after onset of illness in Case 2, one strain was isolated. This strain was typed as Coxsackie virus group B type 3. Serum from this patient showed a more than fourfold increase in antibody titers against the homologous strain and the Coxsackie group B type 3 strain. A summary of the virological data for both patients is given in T ble 1

The virus strains isolated from both patient were tested in cross-neutralization tests against the type strains. Twofold dilutions of rabbit immune serum were tested against 100 TCD_{50} of virus in simultaneous tests. The serum from the Coxsackie B 3 strains neutralized the 2 strains t the same degree while the serum produced from the type strain of Coxsackie B 4 neutralized the type strain in a significantly higher titer than the current strain which could induce antibodies neutralizing both strains t the same extent.

All strains isolated gave spastic paralysis

TABLE 1 *Virological data for the two cases of acute cerebellar ataxia*

+ indicates isolation of indicated virus.

Specimen	Days after onset	Neutralizing antibody titer to: Coxsackie B1	Coxsackie B2	Coxsackie B3	Coxsackie B4	Coxsackie B5	ECHO 6	ECHO 9	Polio 1	Polio 2	Polio 3	Complement-fixing antibody titer: Polio 1	Polio 2	Polio 3	Infl. A	Infl. B	Mumps	Herpes simpl.	Paul Bunnell
Case 1																			
Spinal fluid																			
Feces	6				+														
Throat swab	6				+														
Acute serum	6			320	< 20							< 2	< 2	< 2	< 5	< 5	5	8	< 40
Conv serum I	27	< 20	< 20	320	320	< 20						< 2	< 2	< 2	< 5	< 5	5		
Con serum II	72	< 20	< 20	160	640	< 20						< 2	< 2	< 2	< 5	< 5	5		
Con serum III	133	< 20	< 20	160	1280	< 20	< 20	< 40	< 20	< 20	< 20	< 2	< 2	< 2	< 5	< 5	10	8	< 40
Case 2																			
Spinal fluid	5																		
Feces	5			+															
Throat swab	5																		
Acute serum	5			20									< 2		< 5		40	< 2	
Conv serum I	20	< 20	< 20	1280	< 20	< 20			< 20	80	160		< 2		< 5		40		
Conv serum II	35	< 20	< 20	1280	< 20	< 20							< 2		< 5		40		< 40
Conv serum III	123	< 20	< 20	1280	< 20	< 20							< 2		< 5				
Conv serum IV	178	< 20	20	2560	< 20	< 20	< 20	< 20	< 20	80	160		< 2		< 5		20	< 2	< 40

in mice characteristic of the group B Coxsackie viruses and indistinguishable from the effects of the type strains.

Discussion

The heterogeneous etiology of acute cerebellar ataxia seems now to be well established. In most cases described in the literature the disease has been associated with a virus infection and a number of different viruses have been involved. It is important, therefore that all cases of acute cerebellar ataxia are carefully examined virologically The two cases described in this paper add Coxsackie virus group B to the list of these viruses The first patient represents the more clear-cut cerebellar syndrome with acute onset of taxia and intention tremor diagnosed in a previous healthy child with all signs gradually subsiding and disappearing within 8 days after the onset of the disease

In Case 2 the occurrence of prominent prodromal signs of five days duration and certainly mild but nevertheless unmistakable signs of meningitis together with fever and pleocytosis constitute a syndrome more characteristic of an acute meningoencephalitis. However ataxia and tremor dominated the clinical ppearance Added to this, the presence of the tremor of the eyes—accentuated by voluntary ·e movements, regarded as characteristic of acute cerebellar ataxia [5 18 24]—entitles the case to be incorporated, particularly since the benign course was quite in accordance with the requirements for this syndrome.

No major differences concerning antigenicity and mouse pathogenicity were found between the isolated virus strains and the type strains Both Coxsackie B 3 and B 4 strains were isolated sporadically from the same part of the country at the time the patients were admitted to hospital—but no epidemics were prevalent

Both patients seem to have recovered completely The etiological data available concerning acute cerebellar ataxia are for the time being, too few to draw any conclusions about differences in clinical manifestations and prognosis for the different virus infections. Improved methods of obtaining an accurate etiological diagnosis with the aid of a laboratory should be helpful in this respect

Summary

Two cases of acute cerebellar ataxia associated with Coxsackie virus type B 3 and B 4 are described The heterogeneous etiology of the disease is briefly reviewed and discussed and careful virological examination of all cases of acute cerebellar ataxia is recommended.

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(H J) The Children Hospital
Gä le
Sweden

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CASE REPORT

Acute Poisoning with Butazolidin (R) (Phenylbutazone)

by JØRGEN JUUL

From the Aalborg Kommunehospital, Pediatric Department (Head: Chr. Hansted)

Acute poisoning caused by butazolidin (R) (phenylbutazone) has only been described in a few cases [1, * 5 8 11 16] The poisoning can be serious, even fatal, unless quick and effective therapy is instituted.

We therefore consider it is justified to publish a case of acute butazolidin poisoning in a 15-month-old child and refer to 8 similar cases from the literature

Case History

Fifteen-month-old *female* child, healthy previous t admission, 22 hours prior t admission consumed 10–15 t blets butazolidin (R) corresponding t 2–3 g phenylbutazone The general practitioner who was summoned found the patient unaffected and prescribed purgative cascara sagrada. Approximately 5 hours after the ingestion of the tablets a gradual deep quickening respiration developed and *at the same time the* patient became agitated, irritable and had passing jerky spasms in the arms and legs and also 4–5 periods of vomiting After this she became increasingly lethargic with unchanged deep, rapid respiration.

On admission the patient was agit ted with slight stupor The respiration was deep, proximately 50 per minut Rectal temperature: 36.1 *the pulse strong 140 per minute* The skin was warm, dry and the turgor normal. The liver could be felt two fingers under the right curvature in the medio clavicular line otherwise nothing abnormal was found. Weight was 9 kg

The blood analyses on admission were: Hgb. 12.8 g/100 ml, blood urea 50 mg/100 ml, serum-sodium: 136 mN serum-chloride: 108 mEq/L, serum potassium ...8 mEq/L, standard bicarbonate: 16.1 mMd/L, pH in capillary blood: 7 48, pCO_2: 16 mm Hg

About 2 hours after admission an infusion was commenced through a cranial vein with 180 ml of electrolyte mixture (123 ml isotonic NaCL + 45 ml 10% glucose). After which 30 ml of blood were given and the following 18 hours a total of 600 ml electrolyt mixture (360 ml 5% glucose – 120 ml kalii natri chloridi + 120 ml kalii chloridi c. *glucose) To the latter solution there was* in the beginning added a total of 5 mEq N HCO_3. The treatment with infusion was stopped ca. 24 hours after admission as the necessary fluids could be given by mouth, in the beginning as ordinary fluid diet

In the first samples of urine passed 4 hours after the commencement of the infusion a slight proteinuria (1.3 ‰) and microscopic hematuria was found. The mean hour volume was the following day 10–15 ml, the urine was acid (pH 5.65–6.25).

The general cond tion during the first days improved considerably the patient returned t consciousness, the respiration became normal and the pulse lower At the same time the pCO became normalized

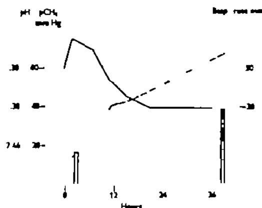


Fig 1 Changes in the serum pH, pCO and respiratory frequence the first 36 hours after admission of a 15-month-old child, who prior t admission had ingested -3 g butazolidin (R). Columns pCO mm Hg. ——— respiratory rate/minute; - - - - serum pH.

whilst the pH in the capillary blood fell to 7.27 (see Fig 1) and the standard bicarbonate increased slightly to 17.3 mMol/L. The proteinuria disappeared 24 hours after d mission, blood urea was normalized after 2 days and the microscopic hematuria ceased on the 3rd day

The concentration of butazolidin (R) in serum[1] was on admisson 12 mg/100 ml.

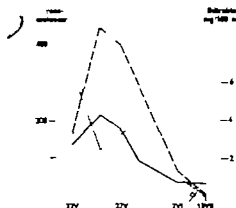


Fig. 2. The course of the serum bilirubin con centration, the SGO and SGP transaminases in 15-month-old child after ingestion of 2-3 g butazolidin (R). serum bilirubin. - - - - SGP transaminase. ——— SGO transaminase

[1] Estimation carried out by Lovens kemiske fabrikker For method see Tophøj [13].

The average urine concentration in the first 24 hours was 100 mg/100 ml corresponding to an excretion of 240 mg butazolidin.

The signs and symptoms of acute poison ing disappeared within the first 2-3 days after which the patient general condition was unaffected, later signs of liver involvement occurred (see Fig 2), with a moderate temporary jaundice On discharge 16 days after admission the jaundice had disappear ed and on ambulant control 5 weeks later the liver tests were completely normal (Fig 2)

During admission the hemoglobin concentration fell to 10.3 g/100 ml, and at the same time the serum iron was found to be 0 036 mg/100 ml. The remaining hematological values (leukocyte- and thrombocyte *and differential count)* were normal also the serum protein and serum electrophoresis were normal

Discussion

The most important data from 8 similar cases of acute butazolidin poisoning published in literature are tabulated in Table I. As in our case the symptoms are mainly localized to the stomach, duodenum, urinary tract and liver Whilst the dyspeptic symptoms are caused by local irritation of the mucous membranes by the butazolidin, the remaining symptoms result from the toxic affects on the parenchyma. To this must be added the functional affect on the kidney tubuli, inasmuch as butazolidin increases the reabsorption of sodium chloride and water (oliguria, weight increase) [9 11 I.] The bone marrow was affected in a single case, however without it being possible to see this in the peripheral blood picture apart from an initial leukocytosis (Case 8 Table 1) All the patients apart from Case 2 in Table 1 were completely cured.

In our patient the picture of poisoning

TABLE 1 *Eight cases of acute butazolidin poisoning Symptoms and pathological laboratory findings*

No.	Ref.	Age (year) sex	B-dose per body weight	Symptoms	Blood analyses
1	[11]	18/1	2 g 11 kg	Proteinuria, hematuria, weight increase (fluid retention)	Acidosis ("alkali reser 16 mEq). Blood urea increased"
2	[11]	1	2 g	Death 30 min. after admission	
3	[8]	4 f	2.6 g	Proteinuria + hematuria, 3rd–8th day; jaundice, 6th–11th day exanthema 9th–18th day	Initial leukocytosis
4	[*]	*0/1	6–3 g	Vomiting proteinuria, jaundice	SGO- and SGP transaminases elevated
5	[2]	20/12	0.8–1.2 g	Vomiting, proteinuria	SGO- and SGP-transaminase levated
6	[1]	2	1.7 g 12 kg	—	Initial leukocytosis
7	[5	28 f.	10 g	Hyperpnoe, anuria, oliguria, polyuria	Initial respiratory alkalosis, blood urea increased
8	[16]	29 m.	10 g	Duodenal ulcus (relapse), macr hematuria 3rd day j undice 3rd–40th day	Initial leukocytosis, bone marrow: toxic degeneration, alk. phosphatases increased

Serum-butazolidin concentration:
1st day: 21 6 mg/100 ml, 5th day: 6 0 mg/100 ml, 8th day: 3 mg/100 ml.
Serum butazolidin concentration:
4th day 8.5 mg/100 ml, 10th day: 4 0 mg/100 ml, 14th day: 0 mg/100 ml.

was dominated the first days by disturbances in the acid base equilibrium resembling the conditions seen in acetyl salicylic acid poisoning in infants [7] This similarity also applied to the patient hyperpnoe which possibly could result from a stimulating affect of the butazolidin on the respiratory center [5]. The respiratory alkalosis was already partly compensated on admission, probably as a result of the effect on the kidney and the liver and a direct acidogenic effect f butazolidin [1]. Gradually the picture became dominated by a metabolic acidosis (see Fig 1) The metabolic component additionally explains the fact that the urine was continuously acid.

The concentration of butazolidin in the serum 36 hours after the ingestion of the tablets was found to be 12 mg/100 ml. The maximum concentration has undoubtedly been higher inasmuch as this already occurs bout 2 hours fter peroral administration [4].

When given in therapeutic doses the butazolidin concentration lies between 5–10 mg/100 ml [3 1., 13]. With concentrations above 10 mg/100 ml the toxicity is considerably increased [15]. This results from the fact that butazolidin in the blood is bound to the serum proteins. When the serum protein has been saturated in concentrations of 10–11 mg/100 ml the excess butazolidin will quickly be

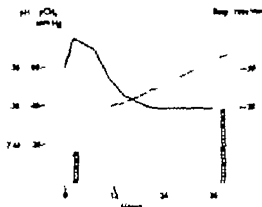


Fig. 1 Changes in the serum pH, pCO_2 and respiratory frequence the first 36 hours after admission of a 15-month-old child, who prior to admission had ingested 2 3 g butazolidin (R) Column: pCO_2 mm Hg. ——— respiratory rate/minute - - - - serum pH

whilst the pH in the capillary blood fell to .27 (see Fig 1) and the standard bicarbonate increased slightly to 17.3 mMol/L. The proteinuria disappeared ..4 hours after admission, blood urea was normalized after days and the microscopic hematuria ceased on the 3rd day

The concentration of butazolidin (R) in serum[1] was on admission 12 mg/100 ml

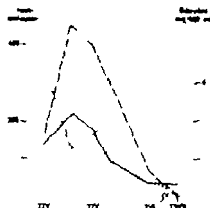


Fig. 2. The course of the serum bilirubin concentration, the SGO and SGP transaminases in 15-month-old child after ingestion of 3 g butazolidin (R). serum bilirubin - - - SGP transaminase ——— SGO transaminase

[1] Estimation carried out by Løvens kemiske fabrikker For method see Tapley [13].

The average urine concentration in the first 24 hours was 100 mg/100 ml corresponding to an excretion of 240 mg butazolidin.

The signs and symptoms of acut poisoning disappeared within the first 2 3 days after which the patient general condition was unaffected, later signs of liver involvement occurred (see Fig 2), with a moderate temporary jaundice On discharge 16 days after admission the jaundice had disappeared and on ambulant control 8 weeks later the liver tests were completely normal (Fig 2)

During admission the hemoglobin concentration fell to 10 2 g/100 ml, and at the same time the serum iron was found to be 0 036 mg/100 ml. The remaining hematological values (leukocyte- and thrombocyte and differential count) were normal also the serum protein and serum electrophoresis were normal.

Discussion

The most important data from 8 similar cases of acute butazolidin poisoning published in literature are tabulated in Table 1 As in our case the symptoms are mainly localized to the stomach duodenum, urinary tract and liver Whilst the dyspeptic symptoms are caused by local irritation of the mucous membranes by the butazolidin the remaining symptoms result from the toxic affects on the parenchyma. To this must be added the functional affect on the kidney tubuli inasmuch as butazolidin increases the reabsorption of sodium chloride and water (oliguria weight increase) [9 11 12]. The bone marrow was affected in a single case, however without it being possible to see this in the peripheral blood picture apart from an initial leukocytosis (Case 8 Table 1) All the patients apart from Case in Table 1 were completely cured.

In our patient the picture of poisoning



TOIVO SALMI †

Professor Toivo Salmi died at the age of 61 on th June His family was Carelian, which dialect he faithfully cherished. H finished his medical studies at the Univer sity of Helsinki in 1932 and as early as that he had also completed his dissertation Experimentelle Untersuchungen über den Atmungtypus bei fruhgeborenen Kaninchen The years at the university were f llowed by a short period of activity in Viipuri, his home town. Subsequently he returned to Helsinki to the famous school of Arvo Ylppö In the 1930 he published several studies on prematures and new borns In 1937 he was nominated Docent of Pediatrics at the University of Helsinki. The war interrupted his scientific work and deprived him of his home county H found new home in Turku where he was appointed the first professor of pediatrics in 1945 Besides his many academi and social duties Toivo Salmi still had vigour

NEW BOOKS RECEIVED

Books received by *Acta Paediatrica Scandinavica* are acknowledged under this heading. Selected books will be reviewed in subsequent issues space permitting.

H WILLI (Ed.): *Symposium über die Frühgeborenen Ernährung* Bad Schachen, /3 Mai 1964 S Karger AG Basel/New York, 1965 326 pages, 115 figures, 48 tables. Price DM 58

DOROTHY CANFIELD FISCHER: *Montessori for Parents* Robert Bentley Inc., Cambridge Mass., U.S.A., 1965 240 pages, illustrated. Price $ 5 95

DOROTHY CANFIELD FISCHER: *The Montessori Manual for Teachers and Parents* Robert Bentley Inc., Cambridge Mass., U.S.A., 1965, 126 pages, illustrated. Price $ 5.

D. A. J TYRRELL: *Common Colds and Related Diseases* Edward Arnold Ltd., London, 1965, 197 pages. Price 42s net

C. H STUART HARRIS: *Influenza and other Virus Infections of the Respiratory Tract*, 2nd ed. Edward Arnold Ltd London, 1965, 248 pages. Price 40s. net

J APPLEY and R. MACKEITH: *Das Kind und seine Symptome in psychosomatischer Sicht.* Translated from English. Hippokrates Verlag, Stuttgart 1965 300 pages.

I A. ABT (Ed.): *Abt–Garrison History of Pediatrics With new chapters on the history of pediatrics in recent times by A F Abt* W B Saunders Company Ltd., London, 1965 316 pages, illustrated. Price £4 0s. 6d.

ANNOUNCEMENTS

International Paediatric Association The American Academy of Pediatrics will hold their annual meeting in Chicago from October 23 to 28, 1965 They cordially invite members of the 11th International Congress of Paediatrics, Tokyo, coming from Europe North Africa, and the Near East, to attend their meeting and will grant them several facilities. I have established with the travelling agency KUONI A G a flight round the world at the price of SFrs. 7000 — (at least 15 participant will be needed) In this amount are included flight (tourist class) and trip by train (first class) accommodation in first class hotels (but no meals in the hotels) Start on October 20 from the airport next to your domicile The trip will include days in New York, 5 days in Chicago (or elsewhere), days in San Francisco. Arrive in Tokyo on November 1 In Japan two excursions by train to Nikko and Kyoto. Leave for Hongkong November 14 2 days each in Hongkong and Bangkok, 2 days in Delhi (possibility for an excursion to Agra). Arrival in Zurich November 2.

Further particulars and programs are obtainable from KUONI A.G Department Verbandsreisen Bahnhofplatz 7 Zurich. Tel. 051/20 34 11

Prof G Fanconi
Secretary General,
International Paediatric Association

The Jefferson Medical College of Philadelphia 10 5 Walnut Street. On Thursday March 24, 1966, through Saturday March 26, 1966, under the auspices of The New York Academy of Sciences, at the Waldorf Astoria Hotel in New York City there will be conference on *paediatric and adolescent gynecology*

Warren P Lang M.D
Co-chairman

Acta Paediatrica Scandinavica 54 511–518 Nov 1965

From the Children Hospital Medical Center the Boston Lying In Hospital, the Departments of Pediatrics and Pathology Harvard Medical School, Boston, Mass., U.S.A., and the Department of Pedia rics, Yale University School of Medicine New Haven, Conn., U.S.A.

Surface Properties of Saline Extracts from Lungs of Newborn Infants

by E. O R. REYNOLDS,[1] M. M ORZALESI, E. K MOTOYAMA, J M. CRAIG and C. D COOK

Recent investigations have suggested that the alveolar lining layer ha surface active properties which make an important contribution to the stability of the ir spaces. This lining layer contains mat rials (phospholipids or lipoproteins) which reduce their surface tensions to very low levels with decreasing surface area [1 6 17 25] Su h substances, which have been collectively called pulmonary surfactant" were demonstrated by Avery & Mead [ ] in saline extracts of lungs from infants weighing more than 1100–1200 g but were not found in the lungs of smaller infants or of any who had died from the respiratory distress syndrome Absence of surfactant was implicated as an important factor in the pathogenesis of the respiratory distress syndrome [2 7].

The data of Avery & Mead [2] suggest that the appearance of surfactant in the alveoli during gestation is rather a sudden event The technique they employed detects surfactant only when sufficient of the material is present in a lung extract to form a film on the surface of the extract within the period of observation. Therefore it seemed possible that small amounts f surfactant might have remained undetected in th lungs of very premature infants and infants dying from the respiratory distress syndrome

Because the time taken for surface active materials to reach the surface of a suspension is partly dependent on their concentrations [20] the longer such a suspension is allowed to stand the more likely are small quantities of surfactant to be detected.

In this investigation the effect of prolonged aging upon the surface tensions of extracts of the lungs of infants w ighing less than 1?00 g and infants dying from the respiratory distress syndrome was observed.

M terial and Methods

Lung tissue was obt ined t topsy which was performed within 4 hours of death and stored t 4 to 10°C After

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[1] receipt of Wellcome Research Tra el Grant Present address, Paediatric Department University College Hospital Medical School, London, W C 1 England.

N significant hange in surface activity was found in lung issue when re-examined after long 1 months of storage

for numerous voluntary functions The Mannerheim League for Child Welfare and the Carelia Association were the most important of them He was awarded decorations and honorary memberships of several associations Toivo Salmi was a unique personality whose congenial appearance held all the numerous characteristics that made him the most colourful and at the same time most unreserved teacher of our faculty He had many friends near and far We all join the words of American condolences: "This is a loss to all of us who work in pediatrics, for Professor Salmi has been a leader in our work for many years—truly a pioneer

Tuomas Peltonen

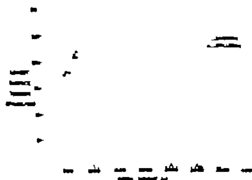


Fig 1

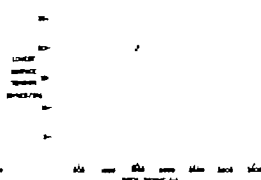


Fig. 2

Fig 1 The lowest surface tension, at about 2½ hours of cycling, of lung extracts from infants dying of causes other than the respiratory distress syndrome vs birth weight. The horizontal line at 15 dynes/cm represents the division between "normal" surface tension (below) and abnormal" surface tension (abov).

Fig 2. The lowest surface tension, at bout 2½ hours of cycling, of lung extracts from infants with the respiratory distress syndrome vs birth weight. The horizontal line at 15 dynes/cm represents the division between "normal" surface tension (below) and abnormal" surface tension (bove) The two X below the line represent measurements from infants recovering from their pulmonary disease (cf. text).

infants or older individuals always have lowest surface tension values below 15 dynes/cm after approximately 2½ hours of cycling [24]; hence extracts with surface tensions below 15 dynes/cm are considered to have normal surface activity

The lowest surface tension of lung extracts from the 30 babies with the respiratory distress syndrome are shown in Fig 2. The surface tension was above 15 dynes/cm, except in 2 infants both of whom died on the third day of life of in-

TABLE 1

Materials	Amount of lung tissue used (g)	Lowest surface tension at 2½ hours (dynes/cm)	Lowest surface tension after prolonged aging (dynes/cm)
Autopsy No. 16099[a]	2.0	5	—
	0.5	10	—
	0.3	22	6
	0.2	24	22
Autopsy No. 16"48[b]	2.0	11	—
	0.8	14	11
	0.6	15	12
	0.4	18	11
	0.2	22	21

[a] An 11 hour old infant who died without the respiratory distress syndrome, birth weight: 3945 g

[b] A 17 year-old healthy girl who died suddenly from traumatic rupture of the aorta.

1–30 days (usually less than 3 days) 2 to 5 g (usually 3 g) of lung tissue were minced in 5 ml of isotonic saline a finely as possible with scissors. This procedure took about 2 minutes. The mixture was then diluted with isotonic saline to 50 ml and stirred vigorously for 2 to 5 minutes. After 20 minutes of standing, the extract was filtered through 4 layers of surgical gauze into the trough of a modified Wilhelmy balance [6]. The surface was aged for 20 minutes and then the surface area was repeatedly expanded to 67 cm² (100%) and compressed t 15 cm (22%) by a motor-driven piston with a 16 minute sine wave cycle. A thin (0 05 mm) stippled platinum bar (4 1 cm) was half immersed in the extract and the weight change of the bar which was proportional to the change in *surface tension, was measured* with a strain gauge transduce Area and surface tension changes were simultaneously displayed on an X-Y recorder and the lowest surface tension reached during cycling was used in analyzing the results.

The lowest surface tension value was obtained when 2 consecutive cycles had recorded the same minimum (within 1 dyne/cm); this usually occurred after bout 2½ hours of compression and expansion of the surface (10 cycles) Measurements were also de on extracts from 3 g samples of lungs m 15 infants after cycling had continued until values below 15 dynes/cm were reached or for 24 hours (i.e up to 90 cycles) In addition the behavior of extracts of varying mount (less than 3 g) of normal lung tissue was studied during prolonged aging. In order to prevent bacterial growth chloramphenicol was added to each extract cycled for longer than 2½ hours. Control experiment showed chloramphenicol to have no apparent effect upon the surface activity of lung extracts.

Lungs from a total of 75 infants were studied. Sixteen of the infants were stillborn, but not macerated, and the remaining 59 died within 4 weeks of birth. Of these 59 30 had evidence of the respiratory distress syndrome as judged by progressive dyspnea and intercostal retraction and, on pathological examination, hyaline membranes and massive atelectasis. In a few infant who died within the first few hours of life hyaline membranes were absent but atelectasis was of the type usually associated with the respiratory distress syndrome. In the remaining 29 infants the pulmonary pathology varied from pneumonia and hemorrhage to normality Of the 15 lung extracts cycled for up to 4 hours, 9 were from infant less than 1200 g 6 of whom were liveborn but died of causes other than the respiratory distress syndrome and 3 of whom were stillborn. The remaining 7 extracts cycled for 4 hours were from infant with the respiratory distress syndrome all of whom were more than 1300 g and had extensive hyaline membrane formation.

Because of the possibility that prolonged aging might result in the formation of plastic membrane of denatured protein which could push th platinum bar up and give a falsely low surface tension reading, the surface activity of plasma proteins was also studied. Plasma (3 ml) serum (3 ml) albumen (250 mg) gamma globulin (100 mg) and fibrinogen (50 mg) in 50 ml of saline were each cycled and the lowest surface tension values read at 2½ and 24 hours.

Results

The lowest surface tensions obtained at about 2½ hours, when 3 consecutive cycles had reached the same minimum surface tension, are shown in Fig 1 and Fig 1 includes only those infants in whom there was no evidence of the respiratory distress syndrome and it can be seen that in babies below 1200 g birth weight the lowest surface tension was above 15 dynes/cm (with one exception) and in those weighing more than 1200 g the surface tension was below 15 dynes/cm (with the exception of one stillborn infant)

With the present technique extracts of 3 g of normal lung tissue from full term

direct information about the behavior of the alveolar surface cannot be obtained.

In this investigation surface tensions below 15 dynes/cm were normally found after about *½ hours of cycling in ex tracts of lungs from infants of more than 1*00 g birthweight (about 28 weeks gesta tion) This result agrees with the findings of Avery & Mead [2] and indicates that the lungs from which the extracts were made contained a sufficient quantity of surfactant for a detectable surface layer to be formed in the trough within ½ hours

The demonstration that low surface tension values can be recorded in extracts of the lungs of very premature infants after prolonged surface aging shows that sur factant is present in small quantities in the lungs of infant weighing as little as 500 g at a gestational age of 23 weeks. It seems, therefore that surfactant first appears in the lungs at about 23 weeks of gestation and increases in mount as term is approached The supposition that if only small quantities of surfactant are present in the trough a long time will be required for low surface tensions to be recorded was confirmed in the xperiments in which very small amounts of normal lung tissue were used to make xtracts The possibility that a plastic surface membrane was formed during prolonged aging which gave a falsely low reading was ruled out by the experiments on plasma proteins.

The finding of surfact nt in very prema ture infants is to be expected. It has been argued that in the absence of surfactant collapse f th lung as seen in th respira tory distress syndrome will occur [*7] It has appeared paradoxical that infant with birthweights well below 1*00 g at an ge when surfactant was previously thought to be absent often live for several days and sometimes survive with no clinical or pathological evidence of the respiratory distress syndrome This was the case in three infants included in the present study who survived 5 1 and *6 days with birthweights of 875 460 and 680 g respectively

Pattle [*6] on the basis of animal ex periments and comparative morphology suggested that surfactant was present from 21–*4 weeks of gestation (at a body weight of about 500 g) onward and Gruenwald [15] demonstrated normal pressure volume relationships indicating the presence of surfactant in the lungs of some infants weighing as little as 300 g Alveolar cells with osmiophilic inclusions which may be the source of surface active ma terials in the lungs [4 7 18 29 30], could not be demonstrated by Campiche in a fetus of 380 g but were present in an infant of 780 g [8]. Our results are therefore consistent with these findings.

Levine & Johnson [19] have shown that saline extracts of degassed normal lungs do not have low surface tensions after *½ hours of cycling on a modified Wilhelmy balance but that after standing overnight low values are reached suggesting that if the lungs are totally collapsed the ex traction procedure does not allow sufficient contact with the alveoli for more than a small quantity of surfactant to be ex tracted Since lung collapse is often almost total in infant dying of the respiratory distress syndrome it might be argued that failure to demonstrate surfactant in this disease is caused in the same way In the present study surfactant could never be demonstrated in lung extracts from in



Fig 3. The lowest surface tension measured at bout ½ hours and after prolonged aging p t 24 hours vs birth weight. The upper of the connected points represent values at about 2½ hours, and the lower values after prolonged aging Where only ne point is shown the lowest surface tension was the same at ½ and 24 hours. The horizontal line at 15 dynes/cm represents the division between "normal" surface tension (below) and abnormal" surface tension (above)

tracranial hemorrhage when there was clinical and pathological evidence of recovery from respiratory distress [5]

The results obtained after prolonged cycling compared with the 2½ hour values shown in Fig 3 Of the 9 infants be- w 1200 g dying of causes other than the respiratory distress syndrome the lowest surface tension after prolonged cycling was still above 15 dynes/cm in the 3 whose birth weights were below 460 g, but was less than 15 dynes/cm in the 6 weighing between 460 and 1100 g The gestational ages of four of these six infants were known and were 22½ 25 26 and 29 weeks respectively The surface tension of lung extracts from the 7 infants with the respiratory distress syndrome remained above 15 dynes/cm.

Table 1 shows the results of the 2 experiments in which the behavior of extracts from small amounts of normal lung tissue was studied during prolonged aging in the trough When 0.2 g and 0 4 g of lung tissues were used the lowest surface tension values at 2½ hours were 22 and 19 dynes/cm respectively but after prolonged aging they decreased to 6 and 11 dynes/cm.

The lowest surface tensions of plasma, serum and plasma protein solutions did not decrease below 20 dynes/cm even with 24 hours of cycling in the modified Wilhelmy balance

Discussion

The surface active materials demonstrated in extracts of minced lung tissue and in lung washings are assumed to be largely derived from the alveolar surface [6 25] Although the method of measuring the surface activity of lung extracts used in this investigation has been frequently employed [2 6 12 19], there are many limitations which must be considered in the interpretation of the results. The amount of lung tissue used, the degree of atelectasis [19] the method of mincing, the time of aging of the surface and the speed of cycling are some of the variables which affect the results. In addition, the minced lung tissue contains substances derived from blood and damaged tissue cells which inhibit the surface layer [1 16 22] so that the surface activity of a lung extract represents the net effect of surface active materials and inhibitors. A further error of unknown, but probably small magnitude results from the variation in the contact angle between the platinum bar and the surface which is assumed to be constant [21] In spite of these difficulties the method remains useful for the measurement of pulmonary surface activity provided it is realised that

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fants with the respiratory distress syndrome (except in the two dying of other causes during recovery) even with 24 hours of cycling. Therefore it is likely that surfactant is completely or almost completely deficient or inhibited in infants dying from this disease.

The reasons for the deficiency of surface activity of lung extracts from infants dying of the respiratory distress syndrome remain incompletely understood. It has been postulated that surfactant might be absent on the basis of biochemical immaturity of the lungs [2]. However, it seems unlikely that the time of appearance of surfactant normally ranges from 23 to 37 or more weeks gestation. In the very premature infant it is possible that surfactant is absent or present in quantities too small to be functionally adequate. In the less premature infant it seems more likely that the small amount of surfactant present is further decreased or inhibited because of damage to the alveolar cells or lining layer by some process such as asphyxia or transudation of fluid from the pulmonary capillary bed into the alveoli occurring before, during or after the time of birth [28]. Asphyxia may injure the alveolar cells directly [28, 29] and also by producing a decrease in pulmonary blood flow [10–12]. Transudation of fluid into the alveoli of infants with respiratory distress which is responsible for the formation of hyaline membranes [13, 14] may be favoured by deficient surfactant [2, 25, 27]. There is also evidence to suggest that the capillaries of premature infants are more permeable to fluid than at full term [3, 9], that asphyxia causes an increase in capillary permeability [31] and that blood elements are capable of inactivating surfactant [1, 16, 22]. The premature infant is therefore particularly vulnerable to the interaction of a number of factors, including deficient surfactant, asphyxia and transudation which are likely to lead to atelectasis and the formation of hyaline membranes.

Summary

The surface activity of saline extracts from the lungs of 76 babies dying in the newborn period has been examined using a modified Wilhelmy balance. When readings were made after 2½ hours pulmonary surfactant could be demonstrated in lung extracts from babies of more than 1200 g birthweight except in those dying from the respiratory distress syndrome. Readings made after prolonged aging of the surface showed that surfactant was present in small quantities in infants with birthweights as low as 500 g. No surfactant could be demonstrated after prolonged aging of lung extracts from babies dying of the respiratory distress syndrome.

It is postulated that the respiratory distress syndrome is caused by a deficit of surfactant in premature infants and that the small amount present is further reduced by alveolar cell damage or inhibited by transudation of plasma into the alveoli.

Acknowledgements

The authors are indebted to Professor E. Merrill and Mr H. Meiselman, Department of Chemical Engineering, Massachusetts Institute of Technology, Boston, for their helpful advice.

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From the Department of Virology University of Turku, and from the Epidemic and Medical Department for Children, Aurora Hospital, Helsinki, Finland

An Outbreak Among Children of Respiratory Illness Caused by Respiratory Syncytial Virus
A Serological and Clinical Study

by BO BERGLUND PER FORSSELL and MARJA HARVO-NOPONEN

Introduction

Epidemics of respiratory tract infection in children due to respiratory syncytial (RS) virus have been described in U.S.A., Europe and Australia and a high frequency of bronchiolitis and pneumonia in young children infected with RS virus during these epidemics has been reported [1-13]

In Helsinki Finland, a widespread outbreak of respiratory disease in children occurred in early December 1963 nd lasted until early February 1964 The high frequency of bronchiolitis and pneumonia among the infants admitted to hospitals suggested that RS virus might be the causative agent.

The present report describes the results of serological studies carried out during the epidemic on hospitalized children with respiratory disease and also the clinical findings in cases of serologically confirmed RS virus infection.

This study was supported by grants from the Sigrid Jusélius Foundation, and the Finnish Medical Research Council.

Material and Methods

Clinical material

A total of 160 children were studied, divided into a respiratory and a control group.

The respiratory group consisted of 119 children under years of age with respiratory disease admitted to Aurora Hospital, Helsinki, during December 1963 and January 1964. The patients, mostly suffering from a severe respiratory disease were all treated on the same ward, which was intended only for children under years of age with respiratory disease. Chest X ray examinations were performed. White blood cells were counted, and the erythrocyte sediment tion rat determined several times during the st y in hospital.

On the basis of the clinical and X-ray investigations the disease was classified as infection of the upper respiratory tract bronchitis bronchiolitis or pneumonia.

All the children f the respiratory group were given antibiotics during their stay in hospital. Fifty-five were given penicillin, 25 chloramphenicol 10 penicillin followed by chloramphenicol, and 10 were given other ant biotics.

The control group consisted of children without respiratory disease treated on different wards of Aurora Hospital and of 4

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(E. O R. R.) University College Hospital
Medical School
Paediatric Department
Obstetric Hospital
Huntley Street
London, W C 1
England

TABLE 1 *Distribution according to age and diagnosis of 59 patients with 4 fold or greater rise in RS virus complement fixing antibody titre.*

Age (months)	No. of patients: Pneumonia	Bronchiolitis cum pneumonia	Bronchiolitis	Bronchitis	Upper respiratory infection
< 3	2	—	1	—	—
3 < 6	8	8	—	2	1
6 < 12	13	7	1	2	3
12 < 24	7	1	—	1	2
Total	30	16	2	5	6

fall in 1 case. All 11 children were over 6 months of age.

Among the controls at the children's home an outbreak of respiratory disease started towards the end of and shortly after the study. Five children of the home contracted a respiratory disease during the time between collection of the first and the second serum specimen. Of these 5 controls a 1 month-old girl showed a rise in titre from less than 1 in 4 to 1 in 8 and an 8-month-old boy a 32 fold rise in RS virus CF antibody titre (Table 1).

During the epidemic serological evidence of RS virus infection in children with respiratory illness was also obtained from another children's home in the town indicating that the epidemic caused by RS virus was widespread.

RS virus isolations

Since the hospital and the virus laboratory were located in different cities virus isolation experiments were carried out on a small number of children only. The purpose of the isolation experiments was not to confirm the serological diagnosis but to test the antigenic similarity of the Randall strain of RS virus and the virus strain that was prevalent during the epidemic.

Isolation specimens were obtained from 17 children of the respiratory group. Virus was isolated from 6 children. All isolates produced syncytial degeneration in cell cultures. The isolates were neutralized by RS virus-immune serum prepared against Randall strain according to a method described elsewhere [3]. Each of the 6 children from whom RS virus was isolated showed a 4-fold or greater rise in CF antibody titre against the Randall strain of RS virus.

Distribution of patients according to age diagnosis and rise in RS virus complement-fixing antibody titre

The children with a 4-fold or greater rise in titre are shown in Table 2. Most of them had a lower respiratory tract in

TABLE 2 *Distribution according to age and diagnosis of 12 patients with 2-fold rise in RS virus CF antibody titre or a rise in titre from less than 1 in 4 to 1 in 4*

Age (months)	No. of patients: Pneumonia	Bronchiolitis cum pneumonia	Bronchiolitis	Bronchitis	Upper respiratory infection
3	5	3*	—	—	—
3 < 6	2	—	—	—	—
6 < 12	—	—	—	—	—
12 < 24	1	—	—	—	1
Total	8	3	—	—	1

* A third specimen obtained from one of these 3 patients 30 days after the onset of the disease revealed a 4-fold rise in titre.

children who were residing at a children's home in the town The children of the control group were of the same age and were studied at the same time as those of the respiratory group The age distribution of all subjects studied is evident from Table 1

Collection of serum specimens and determination of complement fixing (CF) antibodies

From the hospitalized children the first specimen was obtained within 24 hours of admission and the second specimen 7–14 days later but in the children's home the interval between collection of the specimens was 21 days.

For the CF test the sera were inactivated at 56°C for 30 minutes, and serial 2-fold dilutions made in veronal-buffered saline beginning from a serum dilution of 1 in 4 CF antigen was prepared in human amnion cells of a continuous line (U cells) infected with the Randall strain of RS virus, which was obtained from Dr David Taylor Robinson Harvard Hospital, Salisbury Wilts., England The U cell cultures were maintained in Eagle's minimum essential medium containing 200 units of penicillin, 200 micrograms of streptomycin, and 0.025 microg of amphotericin B per ml, and supplemented with 5% tryptose phosphate and 5% horse serum. The cultures were harvested at the time of complete degeneration of the cell sheet frozen at −70°C and thawed 10 times, after which the material was titrated against 4 antibody units of an early convalescent phase RS virus-immune serum obtained from a 4-year-old child. Four antigen units and units of complement were employed in each test The CF tests were carried out in Kahn tubes, using 0 1 ml volumes of serum, complement and antigen. After overnight fixation at 4°C 0.2 ml of a 1.25% suspension of sensitized (2 units of haemolysin) sheep erythrocytes were added to each tube and the tubes incubated at 37°C for 45 minutes. The titre was the highest dilution of serum giving 50 greater fixation. Complement serum and antigen controls were included in the tests, as well as a positive control serum. The CF antibody titre of the positive control serum was 1 in 8 or 1 in 16 in each test

Results

Serological studies

A total of 59 children (50%) of the respiratory group showed a 4-fold or greater rise in the RS virus CF antibody titre (Table 1) The acute and convalescent serum titre of those under 3 months of age never exceeded 1 in 8 On the other hand the convalescent serum titre of the children 1 year or older with a 4-fold or greater rise in titre always exceeded 1 in 8. Of all children in the respiratory group with a 4-fold or greater rise in CF titre 54 had an initial titre lower than 1 in 4

Eleven children of the respiratory group showed an initial titre equal to or higher than 1 in 32 but did not show a 4-fold or greater rise in titre The titre of these 11 remained unchanged in 9 cases, a fold rise was noted in 1 case and a 4-fold

TABLE 1 *Four fold or greater rises in respiratory syncytial (RS) virus complement-fixing antibody titre in the respiratory and control groups*

Age (months)	No. of children: Respiratory group	Control group
<3	3/25* (12%)	1/3
3–<6	19/35 (54)	0/9
6–<12	26/39 (67)	1/10
12–<24	11/20 (55)	0/9
Total	59/119 (50)	2/31 (6%)

* Numerator = no. of children with 4-fold or greater rise in RS virus complement-fixing antibody titre Denominator = no. of children studied.

from 6 to 60 mm/hr the average value being 24 mm/hr The mean maximal leu coeyte count was 9890/mm³

Discussion

In the present study RS virus infection was detected by the CF technique (4-fold or greater rise in titre) in 50% of children under 2 years of age with respiratory disease hospitalized during an outbreak of respiratory illness. The proportionally lowest frequency of RS virus infections confirmed by the CF technique occurred in the 25 children under 3 months of age of whom only 3 showed a rise in titre from less than 1 in 4 to 1 in 8 indicating RS virus infection. Five children under 3 months of age and 2 children of the age group 3 months–<6 months developed a rise in titre from less than 1 in 4 to 1 in 4 suggesting but not proving RS virus infection. If the serum specimens are obtained within 1– weeks after admission to hospital as in the present study these findings indicate the desirability of starting the titration of sera of children under 6 months but especially under 3 months of age from a dilution of 1 in 2 or 1 in 1 instead of 1 in 4

Ross et al. [11] have recently pointed out the importance, for obtaining optimal results by the CF technique of collecting convalescent serum after the 2nd but preferably between the 4th and 6th weeks of illness. In the present study children under 3 months of age failed to respond with 4-fold rise in titre 2 weeks after the beginning of the illness, but showed a 4-fold rise in titre from less than 1 in 4 to 1 in 8 later on, in specimens obtained on the 30th and 32nd day of disease respectively These findings, in accordance with the results of another study [3] carried out in Turku Finland, suggest that children under 3 months of age do not respond with a 4-fold or greater rise in titre from less than 1 in 4 to 1 in 8 or to 1 in 16 until 3 to 4 weeks after the onset of the disease

The occurrence in the present study of respiratory disease in children over 6 months of age with an expected evident antibody response but nevertheless with no rise in RS virus antibody titre indicates that some of the patients studied were suffering from disease caused by agents other than RS virus. The occurrence of laryngitis in children over 6 months of age with no rise in titre for RS virus, together with the absence of laryngitis in children with serologically confirmed RS virus infection, likewise suggests that other agents were responsible for the disease of at least some patients.

Lower respiratory tract involvement (cases of bronchitis included) often in association with severe respiratory symptoms, was found in not less than 90% of the children with serologically confirmed RS virus infection

Four fold or greater RS virus CF antibody responses were detected significantly more frequently in children with respiratory illness than in a control group of children, despite the fact that during the study respiratory infections and subsequent RS virus CF antibody rises also occurred among some controls.

Summary

A serological study was carried out on children under years of age with respiratory disease hospitalized during an out

TABLE 4 *Distribut[illegible] according to age and diagnosis of 48 pati[illegible] with no rise in RS virus complement [illegible]ing antibody titre.*

Age (months)	Pneumonia	Bronchiolitis cum pneumonia	Bronchiolitis	Bronchitis	Upper respiratory infection
3	9*	3	2	—	—
3–< 6	5	1	3	1	4
6–< 12	6	—	—	1	4
1[illegible] 24	3	—	—	1	3
Total	23	4	5	3	11

* A third specimen obtained from one of these 9 patients 32 d[illegible]ys aft[illegible] the onset of the disease re[illegible]ealed [illegible] 4-fold rise in titre.

fection. Bronchiolitis was common up to the age of 1 year Laryngitis did not occur among the 6 children with upper respiratory tract infection

Table 3 shows the children with a 2 fold rise in titre or a rise from less than 1 in 4 to 1 in 4 Three children under 3 months of age with pneumonia showed a ° fold rise in titre from 1 in 4 to 1 in 8 and ° children over 1 year of age one of whom had pneumonia and the other laryngitis, also showed a ° fold rise in titre from 1 in 64 to 1 in 128 and from 1 in 16 to 1 in 32 respectively Seven children under 6 months of age among whom bronchiolitis occurred showed a rise in titre from less than 1 in 4 to 1 in 4

Table 4 shows the children with no rise in RS virus CF antibody titre In these children pneumonia occurred up to the age of 2 years whereas bronchiolitis occurred only up to the age of 6 months Two children shown in Table 4 with upper respiratory tract infection had laryngitis pseudocrouposa and one had laryngitis

Clinical signs and other findings in 59 patients with serologically confirmed RS virus infection

The children with serologically confirmed (4-fold or greater rise in titre) RS virus infection had been ill for an average of 4 days prior to admission to hospital The total length of the respiratory illness varied from 8 to 21 days, being on the average 13 days. The frequency of the main symptoms are shown in Table 5 Most children with lower respiratory tract involvement had marked breathing difficulties In 43 cases removal of secretions from the pharynx by suction was necessary No fatalities occurred among the children included in the study

Further characteristics of the cases of serologically confirmed RS virus infection were as follows: The fever (higher than 37.5°C per rectum) measured on the ward lasted 3 days on the average and the maximal duration of fever was 7 days The erythrocyte sedimentation rate varied

TABLE 5 *Distribution of symptoms of 59 patients under 2 years of age with serologically confirmed RS virus infection*

Symptoms	No of patients
Rhinitis	31
Cough	58
Laryngitis	31
Dyspnoea	19
Cyanosis	29
Wheezing	13
Impaired general condition	3
Otitis media	1
Conjunctivitis	—
Sinusitis	—
Tonsillitis	4
Vomiting	2
Diarrhoea	

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Accepted F b. 10, 1965

(B. B.) Department of Virology
University of Turku
Kiinamyllynkatu 10
Turku, Finland

break of respiratory illness in Helsinki Finland The sera were tested by the CF technique

Of 110 children with respiratory disease 59 (50 %) showed serological evidence of infection with RS virus. Lower respiratory tract involvement mostly in the form of pneumonia and bronchiolitis was found in 90 % of the children with RS virus infection

The lowest antibody response was demonstrated in children under 3 months of age

For the detection of RS virus infection by the CF technique in children under 6 months and particularly under 3 months of age it was found desirable to start the serum titrations from as low a dilution as possible preferably less than 1 in 4

It was found important not to collect the convalescent serum specimen at least from children under 3 months of age until 3–4 weeks after the onset of the disease

Out of 59 children with serologically confirmed RS virus infection, 54 had an acute phase serum titre lower than 1 in 4

In the present study in which the interval between collection of the serum specimens was 1–2 weeks the convalescent serum titre of children under 3 months never exceeded 1 in 8 whereas the corresponding titre of those over 1 year of age always exceeded 1 in 8

Serological evidence of RS virus infection was found significantly more often in the children with respiratory disease than in a control group

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Fig. 1a and b. Microangiogram and corresponding histological section. The lung is derived from a 4-hour-old infant weighing 1710 g. The corresponding arterial branches are marked with points. Between these there is an open air space lined with hyaline membranes.

Fig. 1c. A partial enlargement from the area indicated in Fig. 1b. Between the air spaces lined by membranes there is collapsed lung tissue which contains many contrast-filled capillaries.

to 4 days and then embedded in paraffin. Sections of 1000 μ thickness were cut and they were x-rayed stereoscopically. To obtain a three-dimensional view of the microangiograms the use of a specially constructed microscope is essential. After the examination of the plates suitable areas from paraffin blocks were sectioned serially using standard histological techniques. Sections of 5 μ thickness were cut and stained with haematoxylin and van Gieson. (For complete discussion of the technique see [6].)

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From the Di ion of Pediatric Pathology at the Department f Pathology
Karolinska Sjukhuset Stockholm, Sweden

The Pulmonary Arterial and Capillary Pattern in Hyaline Membrane Disease

A Microangiographical and Histological Study

by ILMARI LINDGREN[1]

A vascular pathogenesis for the "membranes" of hyaline membrane disease is favoured in the latest papers dealing with the subject [7 9 14] It has been postulated that vascular permeability is increased with transudation of fluid and its subsequent transformation to membranes. The increased permeability is thought to be due to functional changes in the pulmonary small arterioles [13] but no morphological changes have been observed in their walls [5].

Boxie [1] recently described an infant with hyaline membranes in all parts of the lungs except those which were supplied only by aberrant vessels originating from the aorta

The possible role played by the pulmonary vessels in the pathogenesis of hyaline membrane formation still remains undefined. A new approach to a study of vascular architecture of the lung in this condition is the use of stereomicroangiographical technique This method also allows parallel histological study of the anatomy of the alveolar capillaries and their relation to the membranes.

Material and Methods

The lungs of 13 infants wh died with hyaline membrane disease were studied. All had typical linical picture pulmonary -ray findings and histological features. Their birth weight varied between 1220-2800 g. The postnatal survival period for 8 infant was less than 4 hours and for 5 infants more than 24 hours. N congenital cardi vascular o other anomalies ere present. The findings were compared with a control series of 15 cases of simila age and weight who died from other causes.

Ten lung pairs had been stored deep-frozen for up to   years in 10 cases and the other 3 were fresh (0-2 days post mortem). There w   n difference n the vascular structure in these both groups.

A 7.5% water suspension of barium sulphate (Micropaque®) wa injected int the pulmonary artery Th is material is stated to ha e a uniform particle ize less than 0.5 micron. The i jection pressure was kept constant between 70-90 mm Hg and the injection time wa one hour After injection the lung swere f xed n neutral 10% formalin up

[1] Research F llow   Pediatric Pathology supported by grants from the Swedish N tional Association against Heart nd Lung Diseases and the Finnish Medical Council. Present address: Department of P thological Anatomy University of Turku, Turk 3, Finland

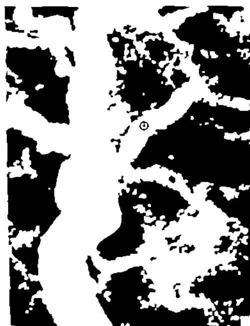






Fig. 1a and b. Microangiogram and corresponding histological section. The lung is derived from 24-hour-old infant weighing 1710 g. The corresponding arterial branches are marked with points. Between these there is an open air space lined with hyaline membranes.

Fig. 1c. A partial enlargement from the area indicated in Fig. 1b. Between the air spaces lined by membranes there is collapsed lung tissue which contains many contrast filled capillaries.

t 4 days and then embedded in paraffin. Section f 1000 μ thickness were cut and they were x-r yed ster-oscopically. T obtain a three-dimensional ew of the microangiograms the use of a specially constructed microscope is essential. After the xamination of the plates suitable area from paraffin blocks were sectioned serially using standard histological techn ques. Section of 5 μ thickness were cut and stained with haematoxylin and van Gieson. (For complet discussion of the technique see [6].)



Fig. — Lung tissue from a premature of 2060 g weight and 22 hours of age. The open air spaces are lined by hyaline membranes with capillaries embedded in them (arrows).

Results

Microangiographical findings

In general a good filling including capillaries was obtained in all cases. To achieve this, however it is important that the injection is done under pressure control and the injection time is long enough.

Lungs from the 8 infants dying during the first 24 hours of life showed the following microangiographic pattern There were small parts with numerous ramifying capillaries. These portions were of irregular shape Between these there were patches about the same size and shape which contained *no capillary filling* The individual arterioles and capillaries were thick and often tortuous (Fig 1*a*) In three cases there were fairly large parts with intense filling of contrast in alveolar lumina In the lungs of the infants dying after 24 hours the ramifying capillary pattern was more regular and concentrations of contrast material were very rarely seen.

In all cases there was filling of bronchial arteries presumably through capillary anastomoses

Histological findings

The areas showing no capillary filling in the microangiograms were seen to be composed of open and dilated air spaces which were lined with hyaline membranes of varying thickness (Fig 1*b*). On the other hand the areas showing numerous contrast filled capillaries were composed of collapsed parenchyma which contained much contrast material in the capillary lumina (Fig 1*c*) Some of these contrast filled capillaries were close to the hyaline membranes and it could be seen that the capillaries were embedded in the membrane material (Fig 2) The large contrast masses seen in 3 lungs represented escape of contrast medium to the alveoli.

In the lungs of the infants dying after *24 hours there were more open* alveolar spaces with or without membranes There were fewer contrast filled capillaries in this group In this group as well the capillaries were close to or adjacent to the membranes.

Comment

The microangiograms from the 13 infants dying with hyaline membranes were compared with a material examined with the same method and collected at this laboratory for other purposes The com-

trols were derived from cases in the same age and birth weight range but without any pulmonary lesions. Apart from the capillary engorgement which was characteristic of hyaline membrane disease even without any contrast injections [12 15], there was no difference. The filling of the bronchial arteries found in the present series can be considered as normal from comparisons with the control material [11]. The presence of the contrast material in the alveoli in three instances is considered to be an artefact.

The well filled capillaries are seen even with ordinary histological technique. However, the observation in this study that the capillaries were embedded in the membranes seems to be a new one. This close anatomical situation suggests that leakage of plasma possibly occurred through these very capillaries which once may have been in contact with air. Using electron microscopy a swelling of the capillary endothelium has been described beneath the membranes in the early phase of the disease [.., 3]. The size of any possible defect in these capillaries must be less than 0.5 micron, since in the present series no free contrast material was observed beneath the membranes.

If it is true that the membranes are formed by a gradual condensation of alveolar oedema the outpouring of plasma into open alveolar ducts must take place in the early phase of the disease when there are many open capillaries in the lung tissue [10 1* 14].

The possibility that the cause of the capillary engorgement in the early phase of the disease might be due to underdeveloped anastomoses between the pulmonary and bronchial artery systems is not supported by this study.

The roentgenological picture of hyaline membrane disease during life shows areas of disseminated densities arranged in a reticulo-granular pattern [4 8]. This appearance can be understood from the microangiographical observations: wide open air spaces with hyaline membranes alternate with atelectatic areas with capillary engorgement.

Summary

The pulmonary vascular anatomy in hyaline membrane disease was studied by a stereomicroangiographical technique after the injection of contrast material into the pulmonary artery. The method allowed parallel histological studies. The material was composed of lungs from 13 infants who died with hyaline membranes in their lungs.

Except for the capillary engorgement in the early phase of the disease the vascular pattern was considered to be normal since a good filling of capillaries and anastomoses to the bronchial arteries could be demonstrated. The contrast filled capillaries were seen to be embedded in the hyaline membranes, a finding which supports a vascular pathogenesis for the membranes.

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Division of Pediatric Pathology
Department of Pathology
Karolinska Sjukhuset
Stockholm 60
Sweden

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From the Department of Paediatrics, Copenhagen County Hospital Hellerup Denmark

Late Metabolic Acidosis in Premature Infants

Prevalence and Significance

By POUL RANLØV and OLE SIGGAARD-ANDERSEN

Ylppö [23] in 1916 was the first investigator to describe neo-natal acidosis. The topic was later discussed at longer intervals by Marples & Lippard [12] in 1932 Räihä [14] in 1941 Wilson *et al.* [22] in 1948 and others. Branning [4] in 1942 dealt in particular with the special problems of acid base studies in premature infants. These studies aimed particularly at elucidating a relationship between acid base disturbance and other clinical or biochemical symptoms of the most varying kinds. In 1950 however Reardon *et al.* [15] were able to confirm that "it has been fairly well established that well premature infants exist in state of acidosis" Since then, further investigators have examined the acid base statu in premature infant during their first weeks of life [1 3 9 10 13].

Kildeberg [9 10] on the basi f 48_ acid base determinations in 123 premature infants (birth weight less than 2500 g) using the same method a in the present study proposed 4 types of neo-natal acidosis.

1 Respiratory acidosis during the first 4 hours of life

2 Combined respiratory metabolic acidosis during the first few days of life

3 Preponderantly metabolic acidosis, occurring most commonly in connection with respiratory distress syndrome

It is characteristic for these three types of disease that they are closely related to neo-natal asphyxia respiratory distress syndrome (RDS) the occurrence of at lectases, malformations of the heart and other mainly pulmonary (hypoxic) complications in the premature state

4 The fourth type a purely metabolic acidosi occurring in th second to fourth week of lif seems t differ essentially from the other categories of acidosis in that it apparently develops without any con picuous clinical symptoms and has no relation t the com plications of prematurity mentioned above

The present study i an account of experience with so-called lat met boli acidosis in particular

Material and M thod

The original patient material includes 171 premature infant with a birth w ight less than 2500 g. [illegible] of these [illegible] the birth weight (BW) in 12 [illegible] less than 175[illegible] g. The most frequent cause of death was RDS and these 16 patien were [illegible]

ded from the present study Only a few of the remaining 55 patients had neo-natal respiratory difficulties of brief durat on, so that all these infant are included in the present material.

The 55 patients were grouped in the follow ing internationally accepted weight groups for prematures:

..500–2001 g: 22 patient
2000–1501 g: 28 patients
1500–1001 g: 5 patient
under 1000 g: 0 patients.

It was possible however with respect to the occurrence of late metabolic acidosis, to separat the patients into two clearly distinguished groups, viz. infants whose birth weight was more than or less than 1750 g In what follows, therefore this distribution has been preferred for the special purpose in question. Twelve of the 55 "healthy" premature infants weighed less than 1750 g at birth (mean BW = 1450 g) The mean BW f the remaining 43 premature infants was 2060 g

A previous study [5] provided a detailed account of the principles of treatment f llowed in this department As a rule premature infants are treated in an incubator (Isolette[2]) during the first hours or days after birth, and an ttempt is made to start feeding as early as the end of the first 24 hours of lif Diet is individual, but as a general rule feeding is started by giving Mamyaan B a bifidogenic dried preparation of cow s milk. After a few days or one week, there is a gradual hange over to feeding with breast milk or 50% mix of cow milk, both diet being enriched with additional protein in the form of skim milk powder After the first 4–3 days, a daily protein intake of at least 5 g per kilo per 4 hours is aimed at and corresponding to this, a daily intake of pproximately 120 calories per kilo bodyweight (see Fig. 7) To 80 ml mother s milk (or nursing milk), or to 60 ml of 50 % milk mix, is added 20 ml of a solu tion consisting of 40 g of kim milk powder dissolved in water

TABLE 1 *Protein and calorific content in the three types of diet employed.*

100 ml Mamyaan B[1] (16 g/100 ml water):
3.0 g protein and 73 cal.
100 ml breast milk + skim milk powder:
4.2 g protein and 86 cal.
100 ml cow' milk mix + skim-milk powder:
4.4 g protein and 84 cal.

Blood sampling

Blood samples for determination of acid-base status were taken immediately after admission to hospital—as a rule 1 3 hours after birth. In some cases, sampling was repeated 4 hours later and thereafter in the case of all infants, daily until the third or fourth day of lif After the fourth day of lif samples were taken every tw day until discharge from hospit l. Most of the infants were followed in this way for more than 3 weeks. All samples were t ken prior to feeding The blood sampling was performed by heel-stab following arterialization by massaging the heel region, the aim being to obtain a free stream of blood without or with only minimum pressure Screaming as a oided if at ll possible

The blood was collected directly from the site of puncture in two heparinized capillary tubes (Clay Adams[3]) and after magnetic stirring and sealing with wax, was immediately stored in a refrigerator at 4°C, to await analysis. The period elapsing from sampling to analysis rarely exceeded one hour part from the first samples after admission these possibly being stored at 4°C overnight; this procedure was not found to change the measuring results significantly Measuring experiment with standard buffers howed no changes in the pH values after passage through the heparinized tubes used.

Technique of analysis

The measuring technique was that described by Astrup *et al* [2] and Siggaard Andersen [17].

The pparatus used which was set up in the laboratory of the department, was RADIOMETER s microtonometer AMT 1 and

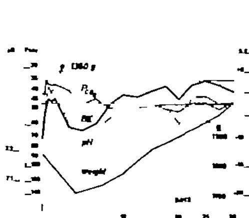


Fig 1

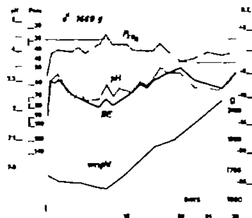


Fig 2

Fig. 1 2. Acid-base parameters in two healthy premature infants showing late metabolic acidosis. (The region between the two horizontal lines indicates the normal range for adults.)

pH-meter 27 The pH-meter was adjusted at every fourth analysis with standard buffer (N tional Bureau of Standards phosphat buffer: pH = 7.384 at 38°C) The temperature of the apparatus was regulated by thermo-st t t 38.0°C.

The actual pH was measured in d plicat and corrected for the patient actual temperature, as was P_{CO_2} At an estimated O saturation of 85% or less (capillary blood), an O saturation determination was performed on capillary blood in a Zeiss PMQ II spectrophotometer following centrifugation and haemolysis at −21°C (18) In these cases, the base excess values were corrected for low O saturation.

The current measuring results were recorded daily f each individual patient on a special "Case History Diagram [17] (see Fig 1–2) In cases of unexpected falls or rises in the base excess values, daily cid

The apparatus was kindly placed at our disposal for this investigation by Messrs RADIOMETER, Copenhagen.

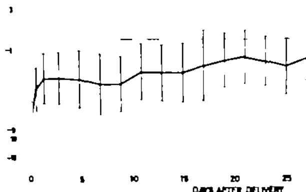


Fig. 3 Mean base excess with standard deviations in 85 healthy premature infants followed from birth. (A total of 892 determinations.)

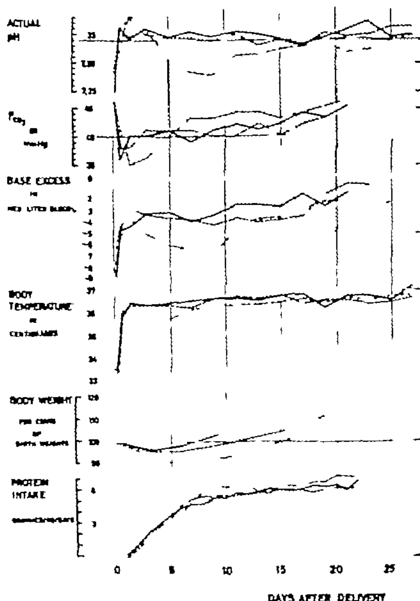


Fig. 4. Acid-base values, temperature, weight, and protein intake in 35 infants with uncomplicated prematurity. —— representing 22 premature infants with birth weights more than 1750 g (mean BW 2066 g), not showing late metabolic acidosis; ---- representing 11 premature infants with birth weights less than 1730 g (mean BW 1440 g) showing late metabolic acidosis; ---- representing 2 premature infants with birth weights more than 1750 g (mean BW 2057 g) showing late metabolic acidosis.

base status determinations were carried out in the individual case

A total of 632 analyses of the acid base st tus were made on capillary blood from the 85 "healthy premature infants included in the present material. Of these analyses 891 were performed within the 4 first weeks of life

Results

By observing the development of the actual pH P_{CO_2} and base excess in each individual patient in the material, a characteristic course for the base excess values could be noted. After the initial preponderantly metabolic acidosis during the first 6 to 10 hours of life often with base excess values of the magnitude of −15 mEq/l, the acidosis decreased to values around −4 or −3 mEq/l, at which level the values stabilized from the end of the first day of life to well into the third day of life Thereafter a falling tendency could be observed an expression of an increasing metabolic acidosis, which in the majority of the cases reached its maximum between the seventh and the tenth day of life then decreasing in the course of 8–10 days. Examples of such a course are shown in Fig 1–2 In a few cases a certain respiratory compensation set in, with a moderate decrease in P_{CO_2} during the days before and after the acidosis reached its maximum. No parallel clinical symptoms, such as dehydration or flaccidity for example could be seen to accompany or precede the acidosis.

Fig 3 shows the mean values and the standard deviations for base excess in the entire material from birth to the end of the fourth week of life The acidoses mentioned above are seen to have involved a temporary fall in base excess between the fifth and the tenth day

Th idoses found were ll degrees and it some cases appeared le a tendency t few days fall in b excess rather th n an actual manifest a idosis. Since th analysis of the materi l made it desirable to be able to oper t with well-defined patient groups *with* met bolic acidosis and *without* metabolic a d sis, respectively the following criteria have been set p in the present study for the selection of premature infants to represent *late metabolic acidosis*

An acidosis occurring after the second day of lif in hich *as a* *m* *m* two consecutive base cess measurement were found t be *at least* 3 mEq l lower than the mean of the initial value and he final value respectively Furthermore the minimum value should be lower than 3 0 mEq l, and the measuring int rv ls should be at least 24 hours.

This somewhat artificial defin tion has proved to be of practical use as by means of it 23 cases with late metabolic acid sis could be selected from the 85 health premature infants included in the material Out of these 23 children 11 were infants with a BW less than 1750 g Thus, of the 1 premature infants in this weight group in the material, 82 showed late metabolic acidosis while the corresponding figure for the 43 infants with a BW more than 1750 g was 28 .

With a view to further analysis, the material was divided into 3 groups

I 32 premature infant with a BW *great than* 1750 g (mean weight 2000 g), *without* lat met bolic acidosis.

II 12 premat re infants with BW *greater than* 1750 g (mean weight 2037 g) *with* late metabolic acidosis.

III 11 premature infants w th BW *less than* 1 50 g (mean weight 1450 g) *with* lat met bolic acidosis.

As only one infant with a BW less than 1750 g did not show late metabolic acidosis this fourth group could not be set up.

Fig. 4 shows actual pH, P_{CO_2} and base excess values, correlated in time with rectal temperature, weight increase and protein intake for each of the three separate groups.

Acid-base status (Fig. 4)

Group I (3.. premature infants with a BW greater than 1750 g *without* late metabolic acidosis). As in the other groups, a pronounced neo-natal combined respiratory metabolic acidosis was found which disappeared spontaneously, however, in the course of a few hours, among other things as a result of a compensatory hyperventilation, with P_{CO_2} reduction at *the end of the first day of life*. From the second day of life the pH values were stabilized at the lower limit of the adult normal range and a slight tendency to a fall in base excess around the seventh day was compensated by slight hyperventilation.

Group II (1.. premature infants with a BW more than 1750 g, *with* late metabolic acidosis). After a combined respiratory metabolic acidosis during the first day and normal acid base conditions in the second day of life, a prolonged fall in base excess set in with a corresponding fall in pH. This relatively weak metabolic acidosis was most pronounced around the ninth day and had quite disappeared on the eighteenth day. It should be noted that the metabolic acidosis in this group showed greater variations in time from one child to another than was the case in group III, which explains the more prolonged and flatter course of the curve.

Group III (11 premature infants with a BW less than 1750 g *with* late metabolic acidosis). This group showed a picture which was identical with that in the two other groups during the two first days of life. Then a pronounced fall in base excess set in, accompanied by a corresponding reduction in pH, in spite of attempts at respiratory compensation during the first few days. The metabolic acidosis reached its maximum on the ninth day of life and had almost disappeared in the course of three days, but had previously been accompanied by a slight hypercapnia, so that the pH was only normalized around the nineteenth day of life.

Temperature, weight and protein intake (Fig. 4)

No immediate temporal relationship was seen between the acid base parameters and rectal temperatures in the three groups. As was anticipated, the temperatures in group III were subnormal for a longer period than in the other groups.

Group I reached a weight minimum on the third day and had regained BW by as early as the seventh day. Group II stagnated at a weight minimum from the third to the sixth day *and did not reach* BW again until the tenth-eleventh day. Group III showed by far the most pronounced (percentage) "physiological" weight loss, as the fall was more steep, and did not reach a minimum until the sixth day. BW was regained only on the fifteenth day of life. Having regained BW all three groups showed a marked increase in weight, most pronounced in group III.

During the first 5–6 days the intake of protein in relation to bodyweight was somewhat less in group III, but for a

period of 3 days after the fifth-seventh day the daily protein intake in this group was approximately 1 g per kilo higher than the protein intake in the other groups, viz. almost 7 g protein per kilo per day. This appreciable protein intake corresponds temporally to the minimum base excess values in group III (the fall in values in the latter group had, however, set in 4 days earlier). A late rise in the protein intake above the level of 7 g per kilo per day for group III around the twentieth day of life was seen once again to correspond fairly well with a slight fall in the base excess values.

The mean values for 10 premature infants in the second month of life (a total of 61 determinations) were: Actual pH [illegible] (± 0.040), P_{CO_2} 44 mm Hg (± [illegible]), and base excess −1.6 mEq/l (s = 1.9).

Discussion

In the present material, the occurrence of a late metabolic acidosis could be demonstrated in just under half of the material of "healthy" premature infants. As a rule acidosis sets in on the third to the fourth day of life, reaches a maximum on the ninth-tenth day and has more or less disappeared by the end of the third week of life. It is uncompensated. The incidence increases with falling BW just as its degree is most pronounced in the lowest weight group. It is not accompanied by striking clinical symptoms, but nevertheless seems to be correlated with a certain stagnation in the weight increase. It is probable but not proven, that a diet with a very high protein content is of significance.

On certain points, therefore, the results reported by Kildeberg [10] have been confirmed. In the present material, and on the present criteria, the incidence of late metabolic acidosis is 41.8 % of 55 infants with a mean BW of 1940 g. In Kildeberg's material (mean BW 1854 g) the incidence is reported to be only 5.6 % and the metabolic acidosis can have its onset as late as the fourth week of life. There may be several reasons for the difference. Kildeberg's blood samples were not collected at regular intervals and only once or twice in approximately 1/3 of his material, so that the percentage mentioned may well be higher. Furthermore, the intake of protein seems to have been considerably less in Kildeberg's material than in the present study.

Reardon *et al.* [18] determined pH, total CO_2 and P_{CO_2} on arterial blood in 9? premature infants from 1 to 65 days old. In none of these infants were all the acid-base parameters within adult normal range.

Blystad [3] determined pH, total CO_2 and P_{CO_2} in capillary blood in 9 premature infants without clinical complications. In agreement with other authors [4, 14, 22, 23], [illegible] be found a tendency to uncompensated [illegible] acidosis, but the measuring results showed considerable spread. Alvarez [illegible] *et al.* [1] followed 57 healthy premature infants from birth to the [illegible] day of life by means of acid-base status [illegible] minations in venous blood. The authors conclude that premature [illegible] born with a very labile acid-base [illegible] in the form of [illegible] metabolic acidosis, which [illegible] with the development of a more [illegible] is replaced by [illegible]. The authors consider the acidosis as a "physiological" phenomenon in otherwise healthy premature infants.

The aetiology and pathogenesis [illegible] metabolic acidosis in premature [illegible] cannot be regarded as [illegible].

discussion of these problems was started in 1941 by Räihä [14] who assumed that it was the result of a preponderantly anaerobic metabolism ("fixed acids") In very convincing tolerance tests, Tudvad *et al* [20] found that the bicarbonate reabsorption in the kidneys was normal, and that an ingestion of bicarbonate could eliminate the "physiological" acidosis in "healthy" premature infants. The experiments of these authors tell in favour of the theory that the cause of the acidosis must be sought in an accumulation of organic acids, the elimination of which requires an ammonia and titratable acid excretion greater than that the premature kidney can achieve this assumption is supported by results with ammonium ion tolerance tests, carried out by Gordon *et al* [8]

In the present material as mentioned, a metabolic acidosis was found most frequently among premature infants with the lowest birth weight. This was also the case in Alvarez de los Cobos material [1] while other authors [9 10 13 15] were unable to demonstrate any certain relationship between acid base status and birth weight or age It should be mentioned however that none of the conclusions which these 4 authors arrive at are based on systematic investigations on the *course* of the acid base variations in each individual patient in the material

All published studies agree on the absence of any relation to clinical conditions and prognosis, apart from variations in the rate of weight increase Studies on a possible relationship with the serum level of sodium chloride glucose and urea, for example have not led to any conclusions [13].

Particular interest is attached to nutrition, mainly on account of the potentially acidosis-producing effect of the protein metabolites (phosphorous- and sulphur containing amino acids) In the present study protein intake was relatively high, as it also was in the material published by Alvarez de los Cobos and coworkers [1], who obtained results which in several respects resemble the present ones

On the basis of nitrogen balance experiments Levine [11] recommended that protein should be administered to premature infants between one and four weeks old, in amounts from 4 4 to 0 0 g per kilo per day—the lower the BW the higher the intake Darrow *et al* [6] produced severe acidosis, influencing the general condition, by an intake of more than .0 g of protein per kilo per day These patients developed Kussmaul respiration, became dehydrated, lost weight, and their pH fell as low as 7 01

Goldman *et al.* [7], in a critical comment on Darrow s *et al* study mention that the protein rich milk had been produced by precipitation with calcium chloride with a further addition of lactic acid. The significance of this will appear from a study by Goldman *et al* from 1961 in which a group of 16 premature infants, having been fed for a period of -10 days on a mix of cow s milk acidified with lactic acid (Pelargon®) developed a metabolic acidosis with an average fall in pH from 7.37 to 7.23 Furthermore the weight increase within this group was only 6 5 g per kilo daily against 14 0 g per kilo daily in a control group which had received an ordinary milk mix and without any changes in pH These results have recently been confirmed by other authors [16].

Vesterdal [1], on the other hand, found no significant changes in the acid base status in 17 premature infants who had been fed on half-skimmed cow milk acidified with lactic acid (Eledon®). Goldman's et al. material and Vesterdal's material are however not directly comparable for one thing because of weight differences in the experimental periods, as the mean weight in the former material was 1023 g against approximately 2300 g in the latter's material.

B. normal values

It may be concluded from what has been said above that it is not directly possible to establish "normal values" for acid base parameters in premature infants—at any rate not during the four first weeks of life. Some infants show varying degrees of metabolic acidosis with or without respiratory compensation while others show a gradually increasing base excess, approaching adult values in their fourth to fifth week of life. The latter group cannot be said offhand to represent "normal" values, as it does not necessarily differ clinically from the other groups. Figure 3, which represents the entire material, gives an impression of the very extensive spread in base excess values, as the 95% range for the fifth day of life for example, covers from +2 to −10 mEq/l. On the other hand, the unquestionable correlation between metabolic acidosis and low birth weight in particular makes it necessary to avoid any attempt at following the line of least resistance by operating with concepts such as "physiological acidosis". A further account of the entire problem will be published in a study now in progress [10], but it should be pointed out here that the adult normal range cannot be used in evaluating the acid base conditions in premature infants, or for that matter newborn infants in general.

Summary

A total of 652 analyses of acid base status were performed by Astrup's micromethod, in an attempt to follow systematically the variations in this status in 53 "healthy" premature infants during the first four weeks of life.

The occurrence of a late metabolic acidosis could be demonstrated in 42% of these children. As a rule the acidosis started on the third or fourth day of life reached its maximum on the ninth tenth day and had more or less disappeared by the end of the third week of life. The acidosis was uncompensated. Its incidence increased with falling birth weight just as it was most pronounced in the lowest weight group. It was not accompanied by conspicuous clinical signs, but nevertheless seemed to be correlated with a certain stagnation in weight increase. It is very likely but not proven, that a very protein rich diet was of some significance.

Aetiology and pathogenesis are discussed.

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Department of Paediatrics
Copenhagen County Hospital
H Herup
Denmark

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From the Department of Paediatrics, Copenhagen County Hospital, Hellerup, Denmark

Does Food Intake Influence the Acid Base Status in the Premature?

By POUL RANLØV and OLE SIGGAARD-ANDERSEN

A previous study of acid base disturbances during the neo-natal period in premature infants [4] led to the question whether the ingestion of food influences the actual acid base status. In other words: does the time elapsing between meal and testing influence the result in a determination of acid base status?

In order to elucidate this problem the acid base status was determined before and after meals in a randomly selected material of infants with uncomplicated prematurity. The determinations were made under standardized conditions.

Material and Methods

The material comprised 15 consecutively selected premature infants without any other symptoms. The mean birth weight was 1800 g (limits: 1100–2150); seven of the infants were girls. At the time of investigation, most of the children were fed on Mamyean B[?] (bifidogenic, half-skimmed dried preparation of cow's milk), while small number of infants were given mother milk without any extra protein. Eight infants were tube fed. The volume of fluid intake at each meal varied from 10 to 40 ml, mean volume being 30 ml.

Twenty examinations were carried out in order to establish the relationship if any between the acid-base status and the time from meal to testing. The examination was carried out twice in 3 children, and 2 times in one child; in all cases at intervals of at least 3 days. Five blood samples were taken for each examination: 30 min and 5 min *before* beginning the meal, and 15 min, one hour and two hours *after* starting the meal, respectively (the length of the meal being 5–10 min). All determinations were carried out between 9 and 11 a.m.

The samples were taken as capillary blood by heel puncture; they were stored at 4°C and analysed within 2 hours after sampling. The acid-base status was determined in RADIOMETER's microtonometer AMT 1 and pH meter 27. The sampling conditions and the technique of the analysis have been described in detail [4].

Results

Fig. 1 shows graphically the mean values obtained for the actual pH, base excess (B.E.) and carbon dioxide tension (P_{CO_2}). The standard deviation of the mean (S) for each of the three acid base parameters (vertical line) is inserted at each of the 5 tests.

The figure shows that there is a tendency to a rise in the actual pH after 1–hours, and a slight fall in P_{CO_2} after hours, but these changes are not significant. The base excess is unchanged throughout the period. No correlations were found between changes in the acid base value and birth weight, volume of meal or manner of ingestion (tube)

Messrs. RADIOMETER, Copenhagen, kindly placed the apparatus at our disposal for this series of investigations.

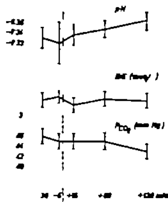


Fig 1 Mean acid base parameters related to the time of food intake Based on 100 determinations in 15 premature infants (S^- is indicated by the vertical line).

A further two premature infants had 30 ml air blown into their ventricles instead of liquid, this did not give rise to any change in the acid base status.

Discussion

In a previous study on late metabolic acidosis in premature infants, an account was given of the long term significance of diet for the acid base conditions in the neo-natal period [4] Studies in adults have shown that immediately after consumption of a large meal there may be a rise in the base excess of the blood, this rise can be of the order of 3–4 mEq/l—the so-called "post prandial alkali tide" [1], while no such effect is seen after normal meals [3 5] This phenomenon is presumably due to variations in the acidity of the gastric juice As the hydrochloric acid production is known to be exceedingly modest in the neo-natal period, and particularly in premature infants [2], post-prandial B E. variations were not anticipated in the present material, and Fig 1 confirms this expectation.

It may thus be considered very unlikely that the intake of normal foods should have any immediate *metabolic* effect on the acid base status in premature infants.

The present investigation was initiated on the assumption that there might be a *respiratory* effect, as the full ventricle, by pressure on the diaphragm could possibly compromise the respiration resulting in increased carbon dioxide tension and transient respiratory acidosis. It appears from Fig 1 that this is not the case

Summary and Conclusion

The acid base status in 15 premature infants (actual pH, base excess and P_{CO_2}) was determined from capillary blood samples taken 30 min and 5 min *prior to* and 15 min one and two hours *after* the start of a meal It is concluded that the acid base status is not significantly influenced by the time elapsing between food intake and testing

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Department of Paediatrics
Copenhagen County Hospital
Hellerup

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From the Institute for the Care of Mother and Child, Czechoslovak Academy of Sciences, Institut of Physiology Prague

Antidiuretic Activity in the Plasma of Human Infants After a Load of Sodium Chloride

by M. JANOVSKY J MARTÍNEK V STANINCOVÁ[1]

It is known that under comparable conditions of dehydration newborn and infant animals are unable to form urine of as high an osmotic concentration as adult animals [8 17] The difference in concentration capacity in the young and in the adult is more pronounced the less mature the newborn. This low concentration capacity has also been described for human infants, and it has been stated that the mean maximum limits of osmolarity in children in the first weeks after birth are about 800 mOsm/L, which means that they only slightly exceed half the level of urine osmolarity in adults [9 18] It is believed that functional and structural immaturity of the hypothalamo-neurohypophyseal system producing antidiuretic hormone (ADH), and insufficient sensitivity of the distal portions of the nephron to ADH are involved. The level of antidiuretic activity (ADA) in the neurohypophyses of newborn rats and puppies is very low [–, 7]. Even in newborn children the ADA content in the neurohypophysis does not reach the level of adult [10]. In addition, the findings of Rodeck [25 26] indicate undeveloped neurosecretion in the hypothalamus of newborn children. Under normal hydration ADA cannot be identified in the plasma of infant rat before the 23rd day of life [3], and in puppies not before the eleventh day of life [–] Under dehydration or after a NaCl load ADA can be detected in the plasma already in 10-day-old rats and 5-day-old puppies [2, 4] Heller & Hradcová [11] have found ADA in the plasma of normally hydrated children only at the beginning of the 5th month.

In the present work it was enquired whether after an osmolar stimulus ADA can be detected in the plasma of even younger infants. Part of the findings has been published as a preliminary communication [14 20]

Methods

Disposal of an NaCl load

NaCl (0.7 g/kg body weight dissolved in two thirds of the child' normal milk intake) was administered t 50 mature children between the ages of 16 days nd 6 months. The NaCl dose was chosen in such a way as not to allow the relative water deficit resulting from administration of hypertonic solution int the gastrointestinal tract to exceed 5% of the body weight Close be-

[1] Technical assistance V Bahlková and R Boušková.

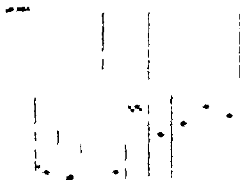


Fig. 1 Antidiuretic activity (ADA) in the plasma of infants before and after administration of a NaCl load. Abscissa: Age of infants in months. Months (long vertical lines) divided into 14-day sections (shorter lines). Ordinate: ADA in μU of vasopressin/1 ml of plasma. O = Individual values before administration of NaCl, ● = individual values 45 minutes after administration of NaCl, -●- = means of individual values in 14-day sections.

fore and 45 minutes after administration of the osmotic load samples of blood were as a rule taken from some veins in the head into heparinized test tubes, and ADA was determined in the plasma. The concentration of sodium and chlorides in the plasma of some of these infants was also determined. To a further 12 infants aged from 16 days to 5 months a NaCl load was applied in precisely the same way as described above; just before and 5, 10, 20, 30 and 45 minutes after administration of milk enriched with salt blood was collected into non-heparinized test tubes, and the Na and Cl concentration, density and dry substance of the serum were determined. In children of this group all values were determined up to 60 and 120 minutes after administration of the NaCl load. These determinations were made with a group of children different from those used for ADA titration in order to avoid great losses of blood and repeated nociceptive stimuli.

Chemical determinations

The Na concentration in the plasma and in the serum was determined by means of a flame photometer Zeiss III.

The Cl concentration was determined by potentiometric titration according to Sanderson [27] in the modification of Drahota & Jelínek [8].

Density of the serum was ascertained in 3 decimal fractions in gradient tubes in a mixture of brombenzene and kerosene prepared according to Lowry & Hunter [19].

The dry substance was obtained by drying a sample of the serum to constant weight at 105°C.

Antidiuretic activity (ADA) determinations

ADA in the plasma was determined in hydrated rats under alcohol anaesthesia according to Heller et al. [13]. One ml of plasma was injected into the tail vein of an adult rat and the percentage of antidiuresis was compared with the percentage of antidiuresis provoked by intravenous injection of vasopressin Parke Davis used as a standard preparation. A single lot of pitressin was used throughout.

The statistical significance of the results was estimated by means of Student's T test.

Results

ADA in the plasma

In normally hydrated infants ADA was first demonstrated at an age of 4½ months (Fig. 1, Table 1). At this time ADA is low

TABLE 1. *ADA 45 minutes after administration of NaCl in μU of vasopressin/1 ml of plasma and concentration of Na in the plasma before (Na_0) and 45 minutes after administration of NaCl (Na_1).*

N	Name	Age	ADA μU/ml plasma	Na_0 mEq/L plasma	Na_1 mEq/L plasma
1	R.	20 d.	0.0	141.–	148.0
2	P.	12 d.	0.0	133.1	152.0
3	Pr.	22 d.	0.0	140.–	144.1
4	K.	4 d.	0.0		
5	M.	4 d.	0.0		

Table 1 (continued)

No.	Name	Age	ADA µU/ml plasma	Na mEq/L plasma	K mEq/L plasma
6	B.	26 d.	0.0		
7	S.	28 d.	0.0		
8	Ro.	30 d.	0.0		
9	Mo.	30 d.	1 1	133.0	145.5
10	Sa.	1 mo. 2 d.	0.5		
11	H.	1 mo. 6 d.	0.0		
12	Z.	1 mo. 9 d.	0.0	128.0	141.0
13	P	1 mo. 12 d.	0 0	137.5	143.5
14	Ph.	1 mo. 12 d.	0.0		
15	Zd.	1 mo. 15 d.	4.0	141.0	157.0
16	Ki.	1 mo. 18 d.	0.0		
17	Pr	1 mo. 19 d.	0.0		
18	Se	1 mo. 20 d.	2 0		
19	Mo.	1 mo. 21 d.	0.0		
20	B	1 mo. 22 d.	0.0		
21	Zi.	1 mo. 22 d.	0.0		
22	W	2 mo.	0 0		
23	Mr	2 mo.	0 0		
24	P	2 mo. 2 d.	0.0		
25	Pa.	2 mo. 3 d.	0.0		
26	Po.	2 mo. 12 d.	0 0		
27	Pe.	2 mo. 3 d.	0 0		
28	Zd.	2 mo. 7 d.	0.5		
29	J	2 mo. 15 d.	1 0		
30	Sm.	2 mo. 15 d.	0.0		
31	Ja.	2 mo. 16 d.	6.0		
32	Jo.	2 mo 19 d.	2.5		
33	Jh.	2 mo. 19 d.	2.2		
34	B.	2 mo. 23 d.	2.8		
35	Ha.	2 mo. 23 d	4.0		
36	Me	2 mo. 27 d.	2.6		
37	Bo.	2 mo. 29 d.	0.0		
38	L.	3 mo.	0 0		
39	Kn.	3 mo. 1 d.	0 0		
40	Ka.	3 mo. 2 d	0.5		
41	No.	3 mo. 5 d.	1.0	129 0	142.0
42	K.	3 mo. 4 d.	2 0	137 6	140 5
43	A.	3 mo. 11 d.	2 0		
44	Ju.	3 mo. 15 d.	2.3		
45	H	3 mo. 20 d.	0 0		
46	Mk.	3 mo. 21 d.	2 5	141.0	141.8
47	V	3 mo. 21 d.	3.5		
48	L.	4 mo. 8 d.	0 6	135.5	140.5
49	T	4 mo. 13 d.	4 5	146 2	153 0
50	Sa.	4 mo. 26 d.	0.5		
51	Se	4 mo. 26 d	4.0		

attaining a value of about 1 µU/1 ml of plasma. After a NaCl load the presence of ADA can be established only quite exceptionally in infants younger than 2½ months; but ADA appears under these conditions regularly in infants older than 2½ months (Fig 1 Table 1) The mean of 6 individual values of the Na concentration in the plasma showed a rise by 11.2 mEq/L in 2½ month-old infants, and by 9.2 mEq/L in infants older than 2½ months (Table 1)

Concentration of Na and Cl density and dry substance values of serum

The values given in Table 2 show that up to 90 minutes following a NaCl load a rise in Na and Cl concentration in the serum is seen in all cases. This increase of *electrolyte content persists for 45 minutes* in all cases i.e until the blood for titration of ADA in the plasma was taken from the second group of infants With the exception of one case (C) the concentration remained elevated for 120 minutes The values of Na and Cl concentration in the serum collected in the 45th minute are

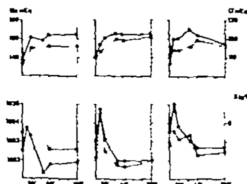


Fig 2. Concentration of Na and Cl in the serum, density and dry substance of serum in infants before and after administration of NaCl. Infant 18 days old (1), 2 months and 8 days old (2), 4 months and 14 days old (3). *Abscissae*: Time in minutes after administration of NaCl. *Top* (Ordinate at the left): Concentration of Na in mEq/L of serum ●—● (Ordinate at right): concentration of Cl in mEq L of serum ○—○ *Bottom*. (Ordinate at the left): density of serum ●—●; (Ordinate at right): dry substance of serum ○—○

TABLE 2 *Concentration of Na and Cl before and after administration of NaCl in the serum Indexes of serum concentration after administration of NaCl. 1 – 5 minutes – 2 – 10 minutes 3 – 30 minutes 4 – 45 minutes 5 – 60 minutes after administration of NaCl 0 – before administration of NaCl*

No.	Name	Age (days)	Na_0	Na_1	Na_2	Na_3	Na_4	Na_5	Cl_0	Cl_1	Cl_2	Cl_3	Cl_4	Cl_5
			mEq/L						mEq/L					
1	Ra.	16	137.4	137.5	148.2	144.0	148.4	146 5	108.6	111 7	120.9	119 4	122.4	129 1
2	C	18	142.5	164.0	14.80	145.2	143.5	140.0	107.0	110 1	—	116.2	113 6	103
3	F	18	140.3	140 0	142.0	142.0	144 1	142.6	102.4	103.2	107 6	116.8	112.8	112
4	P	34	139.2	137.3	141.9	—	148.2	140.5	110 9	103.2	117.8	—	116.3	119
5	H	68	140.5	147.0	150.0	153.0	153.0	154.0	110.3	113.2	—	120.5	119.1	121
6	R.	90	139.6	140.4	—	142.0	—	—	100.0	107 0	—	11_1	—	—
7	W	111	139.5	150 0	153.0	155.5	153.0	—	106.8	117.0	1 1 7	122.4	—	—
8	Pl	134	138.8	146.0	150 0	152.0	15_0	147.5	103.7	114.7	118.8	116.1	119 6	117
9	S	155	139.0	141.6	—	149 6	—	—	102.6	103.2	114._	111.2	—	—
10	W	164	130 0	145.0	138.6	143.2	—	—	104.2	107 1	100 0	103.7	—	—
11	Z	164	141.0	141 0	—	146.5	—	—	103.0	104.5	—	107 6	—	—
12	M	180	139.5	137._	149.0	153.0	—	—	110 1	114.0	119 7	120.9	—	—
13	Zh.	224	141 4	139.6	—	149 4	—	—	100.0	101 4	—	11_8	—	—

higher on the average by 10 0 mEq and 8.2 mEq/l respectively than the initial values (Table 2)

The density of the serum rises within 5–10 minutes after NaCl application by 0 00112 on the average after 120 minutes there is a return to initial values, and later a decrease Similar changes can be found in the dry substance (Table 3) Typical changes in 3 infants of different age can be seen in Fig 2 It follows that in the first stage after administration of a NaCl load a concentration of the plasma takes place while in the second, a dilution occurs.

TABLE 3 *Density (D) and dry substance (D W) of serum before and after administration of NaCl The same indexes as in Table 2*

No.	Name	Age (days)	D_0	D_1	D_2	D_3	D_4	D_5	DW_0	DW_1	DW_2	DW_3	DW_4	DW_5
									g/100 ml					
1	Ra.	16	1 02244	1 02372	1.022_3	1.02126	1 02173	1 0_190	6.37	7 18	6.93	6 07	5.58	5.6
2	C.	18	1 02297	1 02_4 7	1 02285	1 02290	1 02232	1 02200	6.82	7.20	6.31	6 00	7.29	6.4
3	F	18	1 02500	1 02340	1 02370	1 02400	1 02460	1.02480	6.89	7.06	7 47	7 49	6 51	7.9
4	P	34	1 02256	1 02315	1 02218	1 02170	1 02065	1.02264	6.29	6.32	6 73	5 96	6.37	—
5	H.	68	1 02309	1.02360	1.02330	1 02194	1.02194	1 02194	6.16	6.73	6 49	5 77	5.63	5.9
6	R.	90	1.02438	1 02340	—	1.02467	1.02400	1 02400	8 12	8.90	—	8 79	6.18	6.9
7	W	111	1 02433	1 02454	1 02435	1 02366	1 02327	1 02327	7 61	8.10	7.35	7 18	7 09	6.9
8	Pl.	134	1 02336	1 02314	1 02327	1 02300	1 02280	1 02200	6.73	7 84	6.63	6 93	—	—
9	S	153	1.02308	1 02511	1 02414	1 02404	—	—	7 16	8.06	7 73	7 29	—	—
10	Wl.	164	1.02519	1 02560	1 02540	1 02444	—	—	8.23	7 04	6.29	97	—	—
11	Z.	164	1.02244	1 02464		1 02329	—	—	7 92	8.32	—	65	—	—
12	M	180	1.02484	1.02338	1 02520	1 02350	—	—	15	8.96	7.59	8 71	—	—
13	Zh.	224	1.02528	1 02448		1 02446	—	—	8.61	8 30	—	8 14	—	—

Discussion

The present investigation was rendered possible by the use of a very sensitive method which, according to the authors, should allow the determination of ADA *corresponding to 0.025 µU of vasopressin.* But this method does not admit of a continual tracing of ADA so that in our considerations we have to limit ourselves to the interpretation of two fixed values. ADA appears in the plasma of normally hydrated infants at 4½ months at the earliest as found by Heller & Hradcová [11] and in this work. After a NaCl load in infants, on the other hand, the presence of ADA can be demonstrated already in the second half of the 3rd month, in younger infants only quite exceptionally.

Changes in the concentration of Na Cl of density and of serum dry substance have shown that the time course of osmotic and volume stimuli which affect the release of ADH from the neurohypophysis, is the same in all the investigated groups. The changes in dry matter content of the serum may possibly be due to a shift of body fluids into the gastrointestinal tract where a dilution of the hypertonic solution takes place with subsequent absorption. The intensity of osmotic or volume stimuli being equal in all groups, the absence of ADA in most infants up to 2½ months is caused neither by delayed absorption nor by a different distribution of electrolytes in body fluids.

Assuming that the ADA level in the plasma is an indicator of the presence of ADH the results seem to lend justification to the conclusion that the participation of ADH in the regulation of water and electrolytes economy develops in 3 periods characterized as follows.

In the *first* period—up to 2½ months—under normal hydration, it is not possible to identify ADA in the plasma of infants. After an osmotic stimulus ADH is found very rarely. In this period, even exogenously administered vasopressin has a statistically lower antidiuretic effect under water diuresis than in older infants, and does not provoke further excretion of hypertonic urine [23]. Under comparable conditions of dehydration provoked by administration of concentrated milk, 2½-months-old infants furthermore excrete hypertonic urine but of a lower osmolarity than older infants [21].

In the *second* period (approx. up to the beginning of the 5th month) ADA cannot be detected under normal hydration, but is regularly found after a NaCl load.

In the *third* period (beginning with the 5th month) ADA can be demonstrated in the plasma even in normal hydration. It can be assumed that the appearance of ADA is probably an expression of a more mature regulation of water and electrolytes economy which might have gained a new level characterized by a lower intake of fluid [13, 15], decreased hydration of the body [6], and decrease of urine flow in this period [16].

This classification is confirmed by the experiment with young rats [3] and puppies [2]. These seem to show that in the earliest postnatal period in puppies the balance between synthesis and release of ADH from the neurohypophysis is not yet developed. This may be the cause of the quick disappearance of ADA from the plasma during dehydration. Our preliminary examinations have shown that 24 hours after administration of concentrated milk ADA cannot be detected in the plasma

of infants younger than 2½ months. Even in such infants however ADA may temporarily appear in the first hours after lowered intake of fluids. This assumption seems to be supported by the findings of Ames [1] and Nikitin [14] who found surprisingly high values of ADA in the urine of children in the first days after birth dehydrated in Ames' observations for 6 hours and in those of Nikitin for 6–8 hours.

Though we could not demonstrate the presence of ADA in the plasma in the first period of postnatal development, we are faced with the fact that young infants excrete hypertonic urine of a mean osmolarity of about 600–800 mOsm/L during dehydration [22]. What then, is the character of the concentration mechanism, and its function? The question is interesting also because participation of a decreased glomerular filtration rate does not seem to be essential in the formation of hypertonic urine [22]. A study of the functional peculiarities and morphology of the infants' kidney focused especially on the morphologic basis of the counter current mechanism is also desirable.

Summary

Antidiuretic activity (ADA) was determined in 60 mature healthy infants from the age of 10 days up to the end of the 5th month just before and 45 minutes after administration of a NaCl load (0.7 gr NaCl/kg body weight) by stomach tube. Detection of antidiuretic activity in the plasma of normally hydrated infants was possible only in infants older than 4½ months of age; following the administration of a NaCl load ADA was detected already in the second half of the third month. At the time when the blood was collected for the determination of plasma ADA after a NaCl load, the concentration of Na in the plasma of all age groups increased by 11.2 mEq and of Cl by 8.. mEq/L on the average. Relations are being discussed between the development of ADA in the plasma and the development of concentration capacity as well as of the antidiuretic effect of exogenously administered vasopressin. *The possibility is considered that hypertonic urine is formed* in infants younger than 2½ months with little ADH being available or even in its absence.

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(J M.) Czechoslovak Academy of Sciences
Institut of Physiology
Prague 6, Fermingova nám. 2.
Czechoslovakia

From the Department of Human Genetics, The University of Michigan Medical School, Ann Arbor, Michigan

Quantitative Variation of the A Antigen at Birth. Its Significance in ABO Hemolytic Disease and in the Infant's Development

by F. J. GRUNDBACHER

ABO hemolytic disease of the newborn poses certain prognostic problems because maternal antibody titer is poorly related to severity of the ensuing hemolytic process. Heterogeneity of anti-A antibodies, as recently discussed by Polley et al. [10], may be one of the factors responsible for this poor association. Another factor may be the quantitative behavior of the A antigen. Infants with ABO hemolytic disease are predominantly of subtype A_1 and not A_2 [5, 15] and the A antigen is stronger in the A_1 than the A_2 subtype. Furthermore, the A antigen of erythrocytes undergoes marked quantitative change during life: for example, in embryonic erythrocytes it is initially weak but increases slowly during fetal life [8]. After birth, the strength of the A antigen increases rapidly during the first few months of life and then at a slower rate until adult values are reached at about 3 years of age or shortly thereafter [4]. Among adults there is still considerable variation within the A subtypes, which is partly hereditary; this latter aspect will be discussed in a forthcoming publication.

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Studies have been conducted to evaluate the strength of the A antigen of erythrocytes as a factor in the etiology of ABO hemolytic disease of the newborn. A previous investigation was concerned with the determination of the strength of the A antigen in families ascertained for having one or more type A infants suffering from ABO hemolytic disease [5]. In 31 such families there were 47 infants with this disease, 45 were subtype A_1 while 2 were A_2, i.e., a highly significant excess of A_1 over A_2. However, antigen strength of the type A_1 fathers was not significantly different from males of similar ages in the general population. The present communication provides data on the amount and the sources of variability in the strength of the A antigen at birth.

Materials and Method

Fresh samples of blood from the umbilical cord of infants born to type A mothers were collected in the Maternity Clinic of the University Hospital in Ann Arbor, Michigan. If the cord blood was type A, a fresh blood sample was also taken from the mother

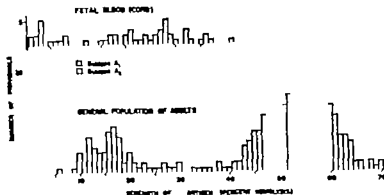


Fig. 1 Frequency distribution of antigen strength among newborn infants and adults (6 years of age and older)

whenever possible. Mother-infant pairs both of type A lessened the probability of the presence of maternal anti-A on fetal erythrocytes, as is likely the case among A infants born to type O and B mothers.

The strength of the A antigen was determined by an immunohemolytic system which was previously described in detail elsewhere (3). Briefly the technique consists of incubating a constant amount of a photometrically standardized cell suspension with a constant amount of hemolytic rabbit anti-human A antibody and complement for 90 min at 37°C. After centrifugation, the density of the hemoglobin in the supernatant fluid is determined photometrically and the percent hemolysis is taken as a measure of antigen strength. Fresh blood samples are essential for reproducible results. Although the subtype of A could generally be predicted from the hemolytic test it was always verified with the lectins from *Dolichos biflorus* and *Ulex europaeus*, as well as human anti A_1. Two infants whose subtype was in doubt were retested at a later date.

The studies were carried out simultaneously with a population study being conducted on antigen strength among adults. This facilitated the experimental procedures because of the essential controls in the quantitative test.

The infant sex, race, birth weight, and duration of gestation were ascertained from the records of the University Hospital only after all the laboratory tests were completed.

Results

The distribution of the strength of 39 A_1 and 14 A_2 cord blood samples are illustrated in Fig 1 along with 289 A_1 and 75 A_2 "adult" individuals (6 years and older) tested in 1963 during a population study. The figure shows that antigen strength of cord cells is markedly weaker than that in adults. The mean antigen strength expressed in percent hemolysis, of 39 A_1 cord samples was 26.0 ± 1.06 while that of 289 A_1 adults was 53.6 ± 0.38. The difference between the two is, of course, highly significant ($P < 0.001$). The mean for the 14 A_2 cord cells was 4.2 ± 0.72 and that of 75 adult A_2 was 17.2 ± 0.66, a difference which is also highly significant ($P < 0.001$). Thus, the A antigen in both subtypes is weaker in cord cells than in corresponding adult subtypes. The strength of the A_1 antigen of cord cells is intermediate to the strength of the A_1 and A_2 of adults, but closer to A_2 with wide overlap in the values of cord A_1 and adult A_2.

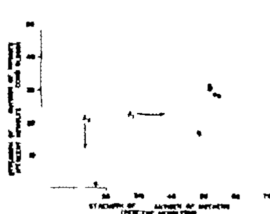


Fig. 2



Fig 3

Fig Relationship between antigen strength in the mother and that of her newborn infant

Fig 3. Relationship between antigen strength of newborn A_1 infants and birth weight

Two A_1B cord blood samples gave hemolysis values of 8 9 and 11 0 %, respectively but these are not included in the figure because the A antigen is known to be depressed in the presence of B

Fig 1 also reveals variation within the A_1 and A_2 subtypes both among adult and cord blood samples The relative variation expressed as the coefficient of variation, is 25 % for the A_1 cord blood samples and 12 % for the A_1 adults Thus variation relative to the mean is twice as large among A cord blood samples than it is among A_1 adults.

The question arises as to the source(s) contributing to variation. The subtypes themselves are major sources of variation and have long been recognized as distinct genetic entities therefore the subtypes will be kept separate in the further analyses.

As stated previously part of the variability in antigen strength within subtypes of type A adults is inherited This can be shown by parent-offspring relationships as between the degree of dependency of the infants antigen strength on that of its mother s Thirty three mother infant pairs were available (7 A_1 and 6 A_2) in which both the mother and infant were of the same subtype These comparisons are illustrated in Fig 2 Numerically the degree of dependence was calculated by regressing the infant s antigen strength on that of the mother For 27 A_1 mother infant pairs the regression coefficient was $b = 0.13 \pm 0.21$ which in light of the large standard error is obviously not significant ($P > 0.05$) This coefficient means that for one unit increase in the mother s antigen strength the infant s antigen strength increased 0 13 units. This low value indicates little association between the strength of the maternal A_1 and the fetal (cord) A_1 However a portion of the infants are expected to have inherited the paternal A_1 and not that of the mother which may partly be responsible for the low degree of mother infant association Nevertheless, it may be surmised that at the time of birth genetic factors contribute only a minor fraction to the total

variation among A_1 infants. This finding prompted the search for other sources of variation, such as sex and birth weight

Since the majority of infants were Caucasians and since racial differences contribute to differences in birth weight [ ] only Caucasians were further analyzed. Occasionally A_2 mothers may form anti A_1 antibodies, therefore no A_1 infants born to A_2 mothers were included. There were 30 A_1 Caucasian infants born to A_1 mothers 18 boys and 12 girls They were all born at term except one girl who was according to menstrual history two weeks early

The average antigen strength, expressed in percent hemolysis, was 25.9 ± 1.57 for boys and 28.4 ± 1.85 for girls Although the average strength was slightly higher for girls than for boys, the difference is not significant ($P > 0.05$) due to the wide overlap in individual values and the relatively small numbers concerned.

The antigen strength according to birth weight is shown in Fig 3 where an association between birth weight and antigen strength is indicated. The magnitude of the association was determined by regression and correlation analyses. The computations were carried out separately for boys and girls because of the usual difference in birth weight The regression equations were $Y = 3.31 + 6.46\ (X)$ for boys and $Y = 9.31 + 5.49\ (X)$ for girls, where X is the birth weight (in kg) and Y is the strength of the A antigen (expressed in per cent hemolysis). Each of the two regression coefficients was positive and approached significance at the 5% level. Since the slopes of the two regression lines were similar and not significantly different from each other ($P > 0.30$) the two sexes were combined by pooling the corrected sums of squares and the cross-products. The regression coefficient for the pooled values was 6.13 ± * 7 This is significantly different from zero ($P < 0.05$) indicating that the association between birth weight and antigen strength is real and not due to chance Hence from the birth weights of infants born at term certain predictions can be made with respect to the strength of the A antigen on fetal erythrocytes.

Similar results were obtained by correlating birth weight and antigen strength the resulting coefficients being $r = 0.43 \pm 0.24$ for boys and $r = 0.33$ for girls. The pooled correlation coefficient (pooled because they were not significantly different from each other) was $r = 0.39 \pm 0.18$ which is significantly different from zero ($P < 0.05$) again indicating an association between birth weight and strength of the fetal A_1 antigen.

The A_2 infants were not further analyzed because the number was relatively small and with the amounts of anti-A and complement used in the immuno-hemolytic system the percentage hemolysis of A_2 infants was low i.e some values very close to the limit of detection.

Discussion

The results presented herein have disclosed that the A antigen in fetal (cord) erythrocytes is weaker than that in erythrocytes of adults. Newborn infants as well as adults manifest considerable variation in the strength of the A antigen both between and within A_1 and A_2 subtypes Although only type A infants born to type A mothers were included in the present investigation, a similar degree of variation

in antigen strength may also exist among infants born to type O and B mothers. This variation in antigen strength appears to be large enough to be of importance in the etiology of ABO hemolytic disease of the newborn because infants with relatively strong A_1 antigen appear more likely to have a portion of their red cells destroyed than infants with a "weaker" A antigen. Furthermore the magnitude of difference in antigen strength between fetal and adult erythrocytes is seemingly large enough to constitute a major protective factor to the fetal red cells against maternal anti-A antibodies that have crossed the placental barrier

The relatively "weak" A antigen and the variation in antigen strength among newborn infants may explain some peculiarities observed in ABO incompatibility such as the observation of Gunson [6] that sensitization of infants cells may occur without clinical manifestation of disease In addition the variation in the strength of the A antigen at birth may explain in part the poor quantitative relationship between maternal antibody titer and of the ensuing hemolytic process. However it is realized that other factors, such as the heterogeneity of antibodies may also contribute to this poor quantitative relationship.

Variation in A antigen strength was previously reported by Fischer [2] but he apparently made no attempt to distinguish between A_1 and A_2 infants or to consider possible effects of antibodies in ABO incompatible pregnancies. With respect to the latter type A_1 infants may display a relatively strong A antigen because of partial sensitization of fetal erythrocytes by maternal anti A antibodies. On the other hand, in a marked hemolytic process the cells first destroyed are likely to have a relatively strong A antigen; the remaining cells could display a relatively weak A antigen Thus, maternal antibodies could contribute to the observed variation in antigen strength among infants of ABO incompatible pregnancies.

Schellong [12] recently reported that premature infants rarely suffer from ABO hemolytic disease despite the presence of immune anti A or anti-B antibodies He interpreted this finding on the basis of "incomplete maturity" of the A and B antigens. Since the strength of the A and B antigens increases continuously during fetal life [8] the damage on fetal erythrocytes by maternal anti-A or anti-B is expected to increase with an increase in duration of pregnancy Thus in ABO incompatibility destruction of fetal erythrocytes by maternal antibodies may be expected to occur predominantly in the latter stages of pregnancy and neonatally This may explain the relatively high frequency of cases of ABO hemolytic disease characterized by hyperbilirubinemia and an absence of early anemia. On the other hand severe early anemia is not an infrequent finding in Rh hemolytic disease However the Rh antigens are not known to undergo marked quantitative change during the individual's life and destruction of fetal erythrocytes by anti Rh antibodies probably begins earlier than in ABO incompatible pregnancies

Of the recognized sources of variation in antigen strength among A_1 infants born at term birth weight showed the only significant effect This effect was considerably larger than that of the maternal A_1 antigen. However parental (or genetic)

effects on variability are more pronounced among adult A_1 individuals than at birth as will be shown in a forthcoming publication. At birth the genetic variation among A_1 individuals may become swamped by the more pronounced effect of birth weight.

The significant association between birth weight and strength of the A_1 antigen suggests that birth weight of infants born at term is more than an expression of size. To my knowledge this is the first instance where an association has been detected between a serological or biochemical characteristic and birth weight among infants born at term. The explanation of this association may be that strength of the A_1 antigen and birth weight both depend on the relative development of the infant born at term. Nevertheless, it is realized that birth weight is only a rough means of assessing relative development; birth weight is known to be influenced by various factors [9-11]. Relative strength of the A antigen from embryo to approximately three years of age depends to a large part on the stage of development of the erythropoietic system and probably the relative development of the infant in general. Elsewhere [4] it was shown that the strength of the A_1 antigen increases very rapidly during the first few months after birth. Hence it seems reasonable that well developed infants measured by birth weight at term are already exhibiting part of the rapid postnatal increase in antigen strength.

Infant girls displayed on the average a slightly stronger A_1 antigen than boys. This is in agreement with the notion that girls develop more rapidly (physiologic, skeletal) than boys of corresponding age despite the fact that the boys exceed the girls in size and birth weight [14]. As suggested elsewhere [5], the difference in rate of development might explain the opposite effect of sex in physiologic jaundice and ABO hemolytic disease. An excess of boys was observed in physiologic jaundice [1-13] while a highly significant excess of girls was found among the infants with ABO hemolytic disease [5]. However, a larger series of cord blood samples will have to be tested for establishing definitely a difference in antigen strength between the two sexes. Unfortunately, testing large numbers of cord blood samples by the immunohemolytic method presents some difficulties—fresh blood samples are required and the number of A mothers who deliver A_1 infants is limited.

Summary

The quantitative aspects of the A antigen of fetal (cord) erythrocytes were investigated by an immunohemolytic system, utilizing 53 type A infants born to type A mothers. Cord cells were found to have a much weaker A antigen than red cells of adults. Marked variation in the strength of the A antigen was also found among and within the A and A_2 subtypes. The variation among A infants appeared to be large enough to be of importance in the etiology of ABO hemolytic disease of the newborn. The analysis of 30 cord blood samples of A_1 infants born at term to A mothers revealed a significant association between the strength of the A_1 antigen and birth weight. The possible implications of these findings on ABO hemolytic disease and development were discussed.

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Department of Human Genetics
The University of Michigan Medical School
Ann Arbor Michigan 48104
U.S.A

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From the Division of Pediatric Pathology t the Department of Pathology (Head. B. Ivemark), the Department of Pediatric Surgery (Head: T Ehrenpreis) and the Department of Gynecology and Obstetrics (Head. U Borell) Karolinska Sjukhuset, Stockholm 60, Sweden

Mucoviscidosis and Intestinal Atresia

A Study of Four Cases in the Same Family

by CARL BLANCK, LUDVIG OKMIAN and HJÖRDIS ROBBE

Observations made during recent years have again directed attention to such prenatal mechanical factors as volvulus, intussusception nd strangulation as the cause of intestinal atresia and the recanalization theory of Tandler [21] and Forssner [10] has been refuted [15 16 17 19] The combination of mucoviscidosis and congenital intestinal atresia has furth er contributed to these etiological discussions [4 5 14, 24] The purpose of this paper is to describe the pathological find ings in a family in which four of five siblings suffered from mucoviscidosis and two of them also had intestinal atresia This familial concentration and the pathological findings support the theory that intestinal atresia in mucoviscidosis is probably secondary to meconium ileus as suggested by Bernstein et al [4] and they furthermore to a certain extent make it possible to date the development of atresia

Evolution of Concept

In the first reports mucoviscidosis was regarded as a disease of the pancreas alone but it has later proved t be a systemic disease involving the majority of the exocrine glands of the body For a review of the subject, see di Sant Agnese [6]. Different authors have stressed the changes in dif ferent organs. It was early demonstrated that a number of newborn infants with atresia or stenosis of the small intestine suffer from mucoviscidosis. Among five cases of meconium ileus Andersen [1] described two associated with intestinal atresia.

In 1960 Bernstein et al. [4] developed a theory of the relationship between mucoviscidosis, meconium ileus and intestinal atresia based on several cases. Similar findings and suggestions were also published by Zuelzer et al. [4], Lelong et al. [14] and Bodian [5] on the basis of single cases. Bernstein et al claim that meconium ileus is probably the cause of cert in atresias of the small intestine. Inspissation of meconium can lead t ulceration of the intestinal mucosa and the pressing of meconium int the intestinal wall to perforation and meconium peritonitis. Meconium granuloma forms t the site of the ulceration or per foration and the ensuing scar formation results in stenosi or atresia. Bernstein et al. suggest that antenatal volvulus of the small intestine in fet ses with meconium ileus is another possible mechanism for the development of treasa of the small i testine but that the antenatal volvulus presumably is a direct consequence of meconium ileus ra

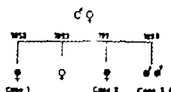


Fig. 1. Genetic chart showing four siblings with fibrocystic disease of the pancreas (black symbols). Parents and fifth sibling clinically healthy (white symbols). The therapeutic abortion not represented

ther than just a coincidence. The statements are largely founded on the fact that among nine cases of atresia of the small intestine they found no less than eight with meconium granulomas in the intestinal wall. In three additional cases with partial intestinal stenosis meconium granulomas also were found in the intestinal wall. These findings and conclusions were confirmed by several authors [6, 9 ..3]. Donnell & Cleland [7] also come to the same conclusion after having discussed the opposite possibility, i.e. that intestinal atresia could be a primary developmental defect followed by meconium ileus.

The frequency of mucoviscidosis in patients with atresia or stenosis of the small intestine is reported rather varyingly. Anderson [ ] gives the number as one in four while Donnell & Cleland suggest 10% [7].

The prognosis of atresia of the small intestine combined with mucoviscidosis is bad. Among Donnell & Cleland nine patients, seven died in hospital within six weeks and the remaining two patients died later in mucoviscidosis. Nine of the twelve cases of atresia or stenosis described by Bernstein et al [4] died very shortly after birth and the reported observation period in the remaining three cases is short.

By this time it is well accepted fact that mucoviscidosis is a hereditary disease. Most authors agree that it is inherited as a recessive autosomal gene [6, 11, 20]. According to this, four siblings with mucoviscidosis in the same family would seem to represent a rather great rarity.

Case Reports

In the family concerned there were five children (see Fig. 1). The mother was ..9 years old at the birth of the first child, the father .0. No consanguinity known.

Case 1. A girl A.N., birth weight 3030 g, born in April 195.. Pregnancy and delivery normal. Immediately after delivery and during the following days attacks of cyanosis. The abdomen was distended from soon after birth and the distension increased gradually. Vomiting from the first day.

Mechanical obstruction was demonstrated radiologically and laparotomy was performed 48 hours after birth. The dilated small intestine ended in an atresia of the middle ileum. The oral segment was extended with inspissated meconium down to the atretic part. On the aboral side of the atresia the small gut was contracted and contained hard masses of meconium. Both the dilated and contracted gut were resected and an anastomosis performed. The resected specimen was not sent for pathological examination.

The immediate postoperative course was satisfactory. However after five weeks the bodyweight was ?650 g, no trypsin activity could be demonstrated in the duodenal secretion and the stools were bulky, pale and frequent. Later on repeated respiratory infections occurred and the infant died at 22 weeks of age with severe respiratory distress.

At *autopsy* the girl was poorly nourished, length 58 cm, weight 2850 g.

All parts of the *lungs* were involved in bronchopneumonia with fibrino-purulent pleuritis, especially on the right side and muco-purulent tracheo-bronchitis. Widespread bronchiectasis in both lungs. Squamous metaplasia was found in a few bronchi. The goblet cells of the bronchial epithelium were prominent. The acini and ducts of the mucous glands in the deeper parts of the bronchial wall were considerably distended with mucus. The hilar lymph nodes enlarged.

Cardiovascular system normal. Ductus arteriosus closed.

Liver not enlarged. Free passage of the bile to the duodenum. No cirrhosis.



Fig. 2. Pancreas (Case 1) with dilated acini filled with amorphous material H & E. 185.

Pancreas firm with granular surface. Moderate interstitial fibrosis. Dilated acini and duct with laminated eosinophilic contents, partly PAS-positive The number of Langerhans islets ppeared to be normal (Fig. 2)

Gastro-intest nal tract: An end to-end anastomosis about 20 cm distal to the duodenojejunal flexure Mesentery normal. N histological sections vailable

Final diagnosis: Cystic fibrosis of the pancreas, atresia of the small intestine with meconium ileus. Bronchitis and confluent bronchopneumonias. Mucoviscidosis demonstrated in respiratory system and pancreas.

Case 2 A girl, birth weight 3000 g, born in November 1956. Pregnancy and delivery normal. At birth distended bdomen and repeated bile-stained vomiting

Laparotomy was performed 24 hours after birth on the diagnosis of mechanical in testinal obstruction. Meconium peritonitis with fibrinous and f brous adhesions and in addition tresia of the ileum was found. The distal part of jejunum and ileum down to the tresia were distended and dark colored but no perforation was visible The distal part of the ileum and colon were contracted. Near the atretic part of the ileum there was a small isolated piece of intestine with a perforation. The gangrenous part of the intestine and the atretic segment were resected and an ileostomy performed. In the resected specimen there was a granulomatous inflammatory reaction with calcification mainly in the subserosa with a foreign body reaction to meconium-like material (Fig 4). No muscular tunics could be demonstrated in sections from the atretic end of the oral segment (Fig 3) A moderately f brotic serosa was directly pposed to a mucosa which contained distended mucosal glands.

The postoperative course was initially favourable From four weeks of age recurrent pneumonias developed a well as septic arthritis in the right knee (Staphylococcus aureus) The stools were large and frequent and no trypsin activity could be demonstrated in the duodenal secretion. The size of the abdomen gradually increased as did the extent of the atelectases and broncho-



Fig. 3 Small intestine (Case). Atretic end of oral segment showing absence of muscular coat The moderately fibrotic serosa encircles the mucosa. Note interruption of lamina muscularis mucosae between arrows van Gieson. 165

pneumonias. The infant died in sepsis at eight weeks f ge

At autopsy the girl was malnourished length 54 cm w ight ..3 3 g

Lu ge Purulent tracheo-bronchitis and ronchitis with bronchopneumonic con 4 lations n both lungs, especiall in the upper ght lobe At lectasis in the upper left lobe B lateral focal nterst tial mph sema and t lectases. Slight distension of the bronchial mucous glands. No squamous metaplasia of the bronchial epithelium

Cardiovascula system normal. Duct is arteriosus losed.

Live not enlarged No fibrosis.

Pancreas macroscopicall normal In the sections there wa marked nterst tial f brosis. A moderat number of dilated acin and duct containing acidophilic substance partly laminated and PAS-posit ve Ap proximately normal amount of slet tissue Few inflammatory cell (Fig 5)

Gastro-intest nal tract Coil f ntesti ies bound together by fibrous adhesions. The anastomosis of the small ntest ne f nger wide Marked dilatation of the stomach, duodenum, jejunum and ileum far as the anastomosis. The rest of the intestines were of normal width with grayish, fluid content In the colon slightly dilated glands.

Right knee swollen with thickened joint capsule and brown contents.

F nal diagnosis Cystic f brosi of the pancreas Atresia of the small intestine Meconium ileu and meconium peritonitis Mucoviscidosis of the respiratory system with bronchiectasis, telectasis, interstitial emphysema and bronchitis and bronchopneumonias. Septicemia.

Case 3 and 4 were premature male twins born in 1938. Hydramnion of 10 liters during pregnancy *del very 3 months before term.* Both infant were alive at birth and died aft one hour The membranes of the placenta were not closely examined.

Autopsy f nd gs (ca 3) Premature boy Length 37 cm. W ight 9.0 g. Bilateral pes equinovarus.

Lungs Bilateral, total at lectasis. Trachea

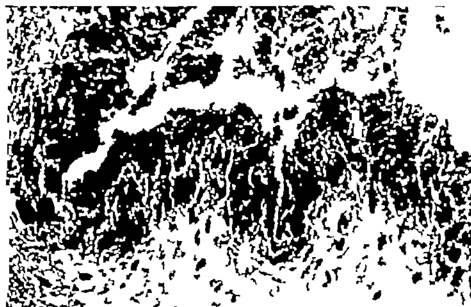


Fig. 4. Case Resected specimen from small intestine. Meconium peritonitis with calcium deposits (dark) and many foreign body giant cells. H & E. ×91



Fig. 5. Pancreas (Case) with dila ed acini, filled with lamellated material. H & E ×185.

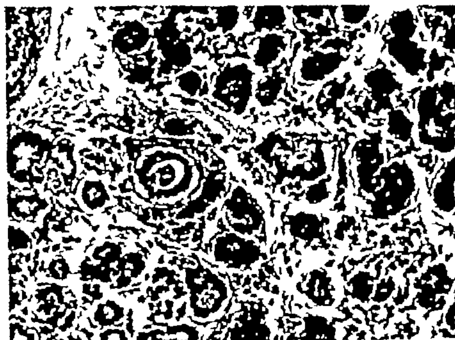


Fig. 6. Pancreas (Case 2) with slightly dilated acini, some of which are filled with inspissated secretion. H & E. ×185

and bronchi normal. Normal mucous gland in the bronchial wall. No squamous metaplasia in the bronchial mucosa. No inflammatory changes.

Liver normal.

Pancreas macroscopically normal. In the section some of the acini were larger than normal and filled with acidophilic material, partly PAS-positive. Slight interstitial fibrosis with lympho-plasmocytic infiltration. The amount of islets was apparently normal (Fig. 6).

Gastro-intestinal system. Common mesentery with malrotation, associated with a volvulus of the major part of the small intestine and meconium ileus. The central part of the volvulus consisted of 5 cm long dilated and darkcolored segment of the ileum. No peritonitis. Distended mucosal glands in sections from the dilated segment of ileum and from the colon (Fig. 7).

Autopsy findings, twin II. Premature boy. Length 3 cm. Weight 7?0 g. Bilateral pes equinovarus.

Gastro-intestinal system. Common mesentery. Volvulus of the small intestine and meconium ileus of the same type as in the twin brother. The other gross and microscopical findings were also identical with those described in the twin brother (Fig. 8).

Diagnoses Cases 3 and 4. Premature twins. Common mesentery associated with volvulus of the small intestine and meconium ileus. Changes compatible with an early stage of mucoviscidosis in the glandular tissue of the pancreas, in the glands of the dilated part of the ileum and to a certain extent of the duodenum and colon. No atresia. No stenosis. No meconium peritonitis.

In February 196–, the mother underwent therapeutic abortion in the 16th week. The fetus was considered to be of female sex and weighed 75 g. Its length was 16 cm. The gross appearance of the organs were normal at autopsy. Microscopic sections from the lungs, liver, kidney, adrenal, pancreas, small intestine and placenta showed no pathological changes. Whether this 16-week old fetal



Fig. 7. Small intestine (Case 3) with dilated mucous glands. H & E. × 72.

Fig. 8. Pancreas (Case 4) with dilated acini containing partly laminated inspissated material in some of the lumina. H & E. Reduced to [illegible] from [illegible] × [illegible]

was affected or not could not be established with morphological methods. To our knowledge the various structural manifestations of mucoviscidosis have not been described before the 8th month of gestation [ ].

Comment

Four of five siblings had mucoviscidosis and meconium ileus and in Cases 1 and 2 in addition atresia of the small intestine. Furthermore, in one of the infants (Case 2) meconium peritonitis could be demonstrated (Fig. 4) with granulomas similar to those described by Bernstein et al. [4] and Donnell & Cleland [7]. The twins (Cases 3 and 4) had identical autopsy findings: meconium ileus, common mesentery, malrotation and volvulus. Common mesentery apparently predisposes to volvulus. The combination volvulus, atresia and mucoviscidosis has been described by Bodian [5] among others. Oppenheimer & Esterley (18) found 20 cases of neonatal intestinal obstruction among 72 cases of cystic fibrosis. Out of these 20 patients *four* had volvulus, three of them also had meconium ileus, and two of these showed stenosis of the ileum. These authors cite 22 cases of meconium ileus with volvulus in newborn children with cystic fibrosis. They state that "volvulus is a relatively *frequent neonatal complication of cystic fibrosis*". The morphological findings in all four cases confirm the theories of Bernstein et al. that atresia and stenosis in the small intestines of patients suffering from mucoviscidosis is probably secondary to intrauterine meconium ileus with or without volvulus.

The fact that bile-stained meconium was found distal to the atresia makes it possible to date with some degree of accuracy the process that led to atresia in Cases 1 and 2. There seems to be considerable disagreement about when the bile secretion begins—from the eleventh week at one extreme to the fifth month at the other [3, 1..., 22]. Whichever view is correct, the finding of bile-stained material in the intestinal lumen distal to the atresia suggests that the process did not take place earlier than the 11th fetal week at least. Furthermore, the twins who could have developed an atresia *on the basis of* meconium ileus and volvulus had reached the degree of maturity corresponding to the seventh fetal month at birth. These facts suggest that the process leading to intestinal atresia or stenosis in mucoviscidosis takes place fairly late.

The diagnosis of mucoviscidosis in Cases 3 and 4 was made from the microscopical appearance of the pancreas compared with that of normal pancreas at the same age and by the changes in the mucosal glands of the intestine (Fig. 7). Of course, the familial concentration directed attention toward mucoviscidosis. The slight morphological changes in the pancreas in these two cases (Fig. 6 and 8) are probably among the earliest changes described in mucoviscidosis (see Andersen [2]).

Landing [13] stated that both the time of onset of the disease and its degree of severity may be regulated by genetic factors. The uniformity of our cases lends some support to this.

Summary

Four of five siblings suffered from mucoviscidosis and two of them were operated upon for intestinal atresia. Meconium

granulomas in the intestinal wall near the atresia and meconium peritonitis were found in one surgical specimen. Two of the infants, immature twins delivered three months before term, died one hour after birth with a common mesentery, malrotation, meconium ileus and volvulus, which probably in due course could have led to intestinal atresia. Our findings support the theory of Bernstein et al. that intestinal atresia in mucoviscidosis is secondary to meconium ileus with or without volvulus. The immaturity and the pathological findings in the twins and the fact that bile-stained meconium was demonstrated distal to the atresia in all the infants suggests that the atresia might have developed at a late stage of pregnancy.

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Division of Pediatric Pathology
at the Department of Pathology
Karolinska Sjukhuset
Stockholm 60

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From the Department of Pediatrics, Karolinska Institutet Crown Princess Lovisa s Children Hospital Stockholm Sweden

The Effect of Physical Activity on the Body Measurements and Work Capacity of Overweight Boys

by B BLOMQUIST M BÖRJESON Y LARSSON B PERSSON and G STERKY

Obesity in childhood is still a major therapeutic problem In previous investigations it has been pointed out that a low level of physical activity might be of significance in the etiology of obesity and overweight [3 7] Subnormal work capacity has been found among overweight children [1] restricting their possibility of taking part in group activities often demanding good physical fitness. Impaired work capacity might be of even greater significance regarding environmental accommodation and social acceptance than overweight per se Thus it seemed of interest to study the effect of physical training on overweight children

In the present investigation a group of overweight boys given extra gymnastic exercise has been compared with a similar untrained group with the main purpose of studying the influence on physical work capacity and also with that of finding out if the increased activity cause changes in bodyweight fatness and caloric intake.

Material

The present series was collected in a w y similar to that of Borjeson [1]. On the basis of special tables, school nurses in the southern region of Stockholm, reported (in October 1962) all boys in class 2 and 3 (aged 8 9 years) who had a weight equal t or exceeding the mean plu twice the standard deviation (sigma) t a given height The height weight tables of Broman et al [*] were used as standards as they appear to be applicable to Stockholm children today [1]. Out of 3**0 male pupils 85 bovs ere reported. Two were late omitted because of incorrect data.

The parents were then asked by letter if they wanted their boys to take part in a physical training program Forty-ei boys were interested, but three had to be excluded because of illness (asthma, epilepsy and stuttering) The remaining 43 were ranked according to increasing level of overweight The odd-numbered boys ere selected for training (22 cases). The even numbered comprised the control group (21 cases). One of the control had t withdra on account of illness and another later refused to take part in the stud In the primary group of 83 boys, height and weight were once more reported by the school nurses in October 1963

Methods

The period of increased exercise as limited to four months F bruary to May 1963. The boys were offered tra gymnastics twice weekly but no effort was made to influence or measure their ordinary daily activity Each lecture conducted by t

especially interested physical education teachers, comprised 45 minutes of intense physical activity steadily increasing in intensity and mostly performed in small, competitive groups.

Immediately before and after the training period, the subjects and controls were submitted t the following measurements: Clinical history and examination, height and weight determination and calculation of the overweight level expressed in sigma. Skinfold thickness was measured by the same person with a caliper [4] at three different sites on the left side: over the center f the triceps, immediately below the tip of the scapula, and midway between the umbilicus and the iliac crest. A mean value for the three measurements was calculated for each patient.

The physical work capacity was determined in the sitting position on an electrically braked bicycle ergometer [5]. These determinations were completed for the whole group in one week. The rat of work in kpm/min which the boys could perform t a heart rat f 170 in relatively steady stat (PWC_{170}) was calculated by means of inter or extrapolation according to Holmgren et al. [6]. Heart volume was determined in the prone position using the method by Kjellberg et al. [8].

The food intake was studied by the method f 24-hour recall (for references see Sterky [9]). The interviews took place at the time f the first two and the last two gymnastic lectures. They were conducted by four experienced interviewers who inquired subject or control selected at random. Neither the boys nor their parents were told of the interviews in advance and intentionally this was the only time during the investigation when diet was discussed.

Results

Individual data on age height weight and skinfold thickness are given in Table 1 for the subjects and in Table 2 for the controls. The mean values (Table 4) show a good correspondence between the groups. The average height of the overweight boys was about 5 cm above that expected for their age.

The history and clinical examination revealed no signs of significance and the ECG was normal at rest as well as during the work tests in all cases.

Table 3 gives individual values for heart volume and PWC_{170}. The mean PWC_{170} for 40 subjects and controls was 111.6 kpm/100 ml heart volume and 12.0 kpm/min/kg body weight. The PWC_{170} per kg body weight or per 100 ml heart volume tended to decrease with increasing overweight.

On account of long travel distances, schoolwork and minor illnesses only three boys could attend all 32 gymnastic lectures. The mean attendance figure was 24 and the lowest 11 times (Table 1).

During the observation period of 4 months the mean increase in height was 0.4 cm higher among the subjects (Table 4). The mean gain in weight was the same in both groups and there was an equal fall in overweight level (Table 4).

The skinfold thickness (Table 1 and 2) showed a decrease at all the measured sites, the most pronounced being below the scapula. A t test between the mean of the individual differences gave $P < 0.01$ among the subjects and $P > 0.05$ among the controls.

The physical work capacity increased 7–8 % in both groups and the heart volume remained unchanged (Table 3 and 4). The mean increase in PWC_{170} among the eleven subjects with the highest initial weight level was +61 kpm/min corresponding to 23 kpm/min among the other ten. Dividing the subjects according to

TABLE 1 *Data concerning the 22 subjects before and after the increased physical act[illegible]*
The cases [illegible]re ranked according t[illegible] increasing level[illegible] of overweight.

Case No.	Age (yrs./mo.)	Height (cm) (before)	Weight (kg) (before)	Weight (kg) (after)	Skinfold thickness (mm) (before)	Skinfold thickness (mm) (after)	Attendance in training (times)
1	8.3	135.5	32.5	35.0	[illegible]	9.1	1
3	10.9	138.5	37.1	38.1	11.8	13.9	15
5	8.7	139.0	37.6	38.2	8.9	10.1	1
7	9.7	137.0	37.1	38.4	14.3	14.2	21
9	9.11	15[illegible].5	5[illegible]	52.4	23.3	20.3	27
11	8.7	140.0	39.7	40.5	1[illegible]9	20.0	22
13	9.3	144.5	45.4	45.0	[illegible]4.8	1[illegible].8	22
15	9.4	141.5	4[illegible].3	42.2	17.8	18.4	4
17	9.7	14[illegible].0	4[illegible]	41.7	1[illegible]9	1[illegible].2	[illegible]
19	10.0	14[illegible].5	44.6	45.1	22.3	[illegible]9.8	[illegible]
21	9.5	137.5	39.1	39.7	[illegible]0.9	17.7	[illegible]
23	9.11	139.0	40.9	40.9	20.2	19.7	[illegible]
[illegible]5	8.1	132.0	34.1	35.3	—	—	[illegible]
27	8.2	134.5	37.6	38.1	18.7	18.3	31
[illegible]9	9.9	135.5	38.1	39.9	17.6	11.2	[illegible]
31	9.3	147.0	50.9	53.4	20.7	19.7	[illegible]
33	8.9	141.0	45.0	45.5	—	—	11
35	10.2	141.5	46.1	49.4	22.7	2[illegible].6	17
37	8.5	137.5	4[illegible].8	45.0	22.8	[illegible]4.6	[illegible]
39	8.6	138.0	42.0	4[illegible].9	22.8	1[illegible].1	22
41	8.6	131.0	38.5	39.7	27.0	[illegible]4.8	4
43	8.2	134.0	44.0	44.2	[illegible]8.9	22.3	13

TABLE 2 *Data concerning the 19 controls before and after the increased physical activity*
The cases are ranked according to increasing levels of overweight

Case No.	Age (yrs./mo.)	Height (cm)	Weight (kg) (before)	Weight (kg) (after)	Skinfold thickness (mm) (before)	Skinfold thickness (mm) (after)
[illegible]	9.2	139.5	36.8	40	10.4	19.3
4	10.4	140.0	3[illegible].8	39.8	1[illegible].3	16.5
6	8.5	137.0	36.6	36.7	16.2	15
8	9.0	140.5	40.1	4[illegible]	[illegible]1	1[illegible].6
10	9.2	141.5	41[illegible]	43.5	16.0	16.2
1[illegible]	9.6	147.5	4[illegible].5	44.7	[illegible]9.9	17
14	10.0	141.5	41.4	—	16.	12.6
16	8.5	135.5	40.3	41.9	19	1[illegible].9
18	9.6	142.0	42.5	42.9	[illegible]9.9	22.1
[illegible]0	10.0	120.0	32.1	31.5	8.9	[illegible]4.9
22	9.9	140.0	41.1	40.0	10.7	1[illegible]
24	9.2	138.0	31[illegible]	40.0	16.9	1[illegible]
[illegible]6	9.2	134.0	36.0	38.4	13.2	13.1
30	10.0	155.0	59.1	59.9	1[illegible].9	1[illegible].9
32	8.5	130.0	33.2	34[illegible]	[illegible]	[illegible]3
34	8.6	143.0	48.0	49.4	[illegible]3	23.7
36	9.10	144.0	49	52[illegible]	19	19.3
38	8[illegible]	130.0	34.9	38.3	31.3	2[illegible].1
40	8.3	155.0	63.6	63.7		—

TABLE 3 *Effect of training on physical work capacity expressed as PWC_{170} and heart volume in subjects and controls*

Subjects

Case No.	PWC_{170} (kpm min) (before)	PWC_{170} (kpm min) (after)	Heart volume (ml) (before)	Heart volume (ml) (after)
1	575	[illegible]	350	390
3	600	530	420	440
5	625	575	350	400
[illegible]	5[illegible]	600	400	370
9	5[illegible]	530	[illegible]	540
11	5[illegible]0	475	[illegible]	[illegible]
13	575	[illegible]	470	410
15	525	[illegible]	440	420
1[illegible]	550	6[illegible]	420	420
19	4[illegible]0	[illegible]	410	430
21	[illegible]00	550	450	410
23	—	525	340	450
25	475	475	430	45[illegible]
[illegible]	[illegible]5	[illegible]	330	340
29	500	625	35[illegible]	[illegible]
31	490	625	410	[illegible]
33	625	450	440	450
35	600	625	35[illegible]	[illegible]
37	400	500	440	460
39	525	60[illegible]	490	4[illegible]
41	600	475	450	340
43	600	700	370	350

Controls

Case No.	PWC_{170} (kpm min) (before)	PWC_{170} (kpm min) (after)	Heart volume (ml) (before)	Heart volume (ml) (after)
[illegible]	[illegible]	6[illegible]	450	[illegible]
4	[illegible]	550	390	3[illegible]
6	4[illegible]	4[illegible]	430	430
[illegible]	5[illegible]	5[illegible]	4[illegible]	[illegible]
1[illegible]	6[illegible]	[illegible]	440	440
[illegible]	6[illegible]	[illegible]	4[illegible]	[illegible]
14	[illegible]	—	[illegible]	—
1[illegible]	[illegible]	[illegible]	410	3[illegible]
[illegible]	[illegible]	[illegible]	31[illegible]	[illegible]
[illegible]	575	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	3[illegible]	450
[illegible]	[illegible]	[illegible]	410	[illegible]
[illegible]	[illegible]	—	[illegible]	[illegible]
3[illegible]	4[illegible]	[illegible]	410	[illegible]
3[illegible]	6[illegible]	6[illegible]	4[illegible]	[illegible]
3[illegible]	6[illegible]	—	[illegible]	[illegible]
3[illegible]	[illegible]	[illegible]	3[illegible]	3[illegible]0
4[illegible]	6[illegible]	[illegible]	[illegible]	[illegible]

actual body weight at the beginning, the figures are exactly the same. Subjects with low initial PWC_{170} showed changes similar to those with a higher capacity. The influence of the intensity of training is shown in Table 5. Favourable data accumulate on the side of those most intensely trained.

Adequate data for analysing the weight development over one year's time were available for 20 subjects, 18 controls and 22 of those initially uninterested in the study. The mean weight level fell from 3 0 to [illegible]8 sigma in the first group, from 3.0 to [illegible]7 in the second and from 3 1 to [illegible]7 in the third. Subjects with a low attendance at the gymnastic sessions did not differ from those with a higher attendance.

The mean caloric intake was initially somewhat higher among the subjects than among the controls (Table 4). Both groups showed a decrease during the observation period which was more pronounced among the trained subjects and also influenced by the intensity of training (Table 5). The mean consumption of protein, fat and carbohydrate before training among the subjects was 70, 94 and 234 g respectively and after 60, 01 and 190 g. Corresponding values for the control group were [illegible] 1, 91 and 224 g and 65, 8[illegible] and [illegible]18 g. No systematic difference could be found between the results obtained by the four interviewers.

TABLE 4 *Mean values before and after training in subjects and control*

		Subjects		Controls	
Age (yrs./mo.)		9.2		9.3	
Height (cm)	before	139 1		140.6	
	after	140.9		14..0	
	diff		+1.8		+1 4
Weight (kg)	before	41 4		4..7	
	after	4..2		43.5	
	diff.		+0.8		+0.8
Weight level	before	3.0		2.9	
	after	2.8		..7	
	diff		−0.2		−0.2
Skinfold thickness (mm)	before	"0 6		19.0	
	after	18.9		18.4	
	diff		−1 7		−0.6
PWC₁₇₀ (kpm/min)	before	489		51	
	after	532		549	
	diff		+43		+37
Heart volume (ml)	before	44		465	
	after	43"		464	
	diff		−10		−1
Calorie intake	before	1"9		"017	
	after	1874		1928	
	diff		−255		−89

Discussion

) The observed frequency 2 0 % of over weight among schoolboys aged 8–0 years corresponds exactly with that reported by Börjeson [1] from a series collected in Stockholm 19.5 As expected the over weight boys had, on the average a height exceeding that of normal boys of the same age The mean heart volume corresponds better to their height than to their age or weight

TABLE 5 *Influence of frequency of attendance at the gymnastic lectures on some parameters*

Mean alues in subject A ttending 4 times (mean 19) and subject B ttending ..5 times (mean 29)

	Subject A	Subject B
Height (cm)	1 8	1.9
Weight (kg)	1.2	0 4
Weight level (sigma)	0..	− 0.4
Skinfold thickness (mm)	0 7	2.3
PWC₁₇₀ (kpm/min)	34	53
Heart volume (ml)	..5	1
Calorie intak	"00	335

Owing to methodological differences in testing physical fitness, seasonal variation etc great care mu t be taken when comparing different investigations The mean figure for PWC$_{170}$ of 1 kpm/min kg body weight in the present series is somewhat higher than that given by Borjeson H w ever the number of cases at each over weight level i different and comparison not fully justifiable A in the presen investigations the physical work capa city per kg body weight or per 100 ml

heart volume tended to decrease with increasing overweight

The 4-hour recall method can only be used for estimating group calorie intake, but even so great caution has to be taken in evaluating the results. In the present study a further drawback is that only two days of the week are represented. In a study employing a similar method and conducted at our clinic in February 1960 [9], the mean caloric intake among normal 7–10 year old boys (mean height 141 cm and weight 32 6 kg) was 2350, as compared with that of .100 (Table 4) in the present series. Our data thus seem to support the findings of previous investigators [7] pointing at a lower instead of the commonly believed higher calorie consumption among obese boys.

The extra training sessions given to the subjects in our study did not cause any dramatic hange in their work capacity or body measurements (Table 4) Our intention was to look into the effect of a training program which interfered with the boys daily life as little as possible Therefore we made no attempt to force the boys to take part Because of absence for legitimate reasons half the group took part only once a week during the four months. When comparing this group with the other half who were able to attend twice a week (Table 5) it is clear that the latter group shows a tendency to less increase in weight, higher decrease in skin fold thickness and higher increase in physical work capacity The training program did not increase the boys calorie intake but instead a small reduction was registered (Table 4) more pronounced among those attending twice a week (Table 5). Whether this is th result of errors of method, an unintentional influence on the boys or their parents by their being under observation or is a true reduction caused by a more physically active life cannot be definitely stated. Against methodological errors speaks unchanged distribution of consumed protein, fat and carbohydrate in the control group. The subjects seem mainly to have cut down their intake of carbohydrates

It is possible that the favourable effect of training is masked to some extent i.e the loss of fat is counterbalanced by a pronounced increase in muscle mass resulting *in a total weight increase* *Our data and* our impression of the boys appearances speaks in favour of such an interpretation but the methods applied are not sensitive enough to justify a statement However even small changes might be of great importance in decreasing obesity if the higher level of physical activity continues over a sufficiently long period of time

The training program, regardless of its intensity did not cause any change in weight development over one year s time

Almost all the boys and parents showed a positive attitude to the study Those who had to be excluded from the training (the control group) were definitely disappointed. The gymnastic teachers were impressed by the boys enthusiasm and willingness but stressed the need for more frequent sessions pointing out that when a boy missed one particular session he was unable to catch up next time Thus the intensity of training could not be increased as qui kly as was desirable It is possible that a training frequen y of three times a week could be obtained at the boy own school and that this would result in a psychologically most important

quicker result both as regards increase in work capacity and decrease in body fat There is no doubt of the great need to take care of overweight boys at an early age and encourage them regularly through the years.

Summary

An unselected group of twenty two 0-year-old boys all weighing above the mean plus two standard deviations for their height took part in weekly extra gymnastic sessions for four months. The influence on physical work capacity body measurements and caloric intake was studied and the results compared with those obtained in a group of non trained overweight boys

No striking differences between the two groups were found. However those who were able to attend twice weekly in the training sessions showed a tendency to decrease more in weight loose more body fat (significant) and increase more in work capacity Initially the overweight boys had probably a lower caloric intake than normal boys of the same age and did not increase their consumption during the training.

It thus seems probable that regular and rather intense physical training is valuable in increasing the physical fitness of over weight boys as well as in preventing obesity

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(G S.) Kronprinsessan Lovisas Barnsjukhus
Polhemsgatan 30
Stockholm K.
Sweden

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From the Hormel Institute, University of Minnesota, Austin, Minnesota, U.S.A.

Polyunsaturated Fatty Acids in Serum of Infants Fed Breast Milk or Cow's Milk[1]

by RALPH T HOLMAN,[2] H. W HAYES,[2] AIME RINNE[3] and LARS SÖDERHJELM[4]

Introduction

If young experimental animals are fed a diet devoid of fat, or containing fat having only saturated fatty acids fat deficiency symptoms appear within a few weeks or months. The more obvious symptoms are a scaly dermatitis, diminished weight gain and changes in the pattern of poly unsaturated fatty acid of tissue lipids. The content of 5,8 11-eicosatrienoic acid increases, and the contents of linoleic and arachidonic acids are decreased. The synthesis of the eicosatrienoic acid from oleic acid does not fully replace the function of arachidonic acid, the principal metabolic product of linoleate Thus, the ratio of trienoic acid to tetraenoic acid has been used as a measure of essential fatty acid deficiency [5], for in deficiency the triene increases and the tetraene decreases. Ratios higher than 0 4 in the tissue lipids of rats and swine have been indicative of essential fatty acid deficiency

The influence of dietary fatty acids upon the polyunsaturated fatty acids of serum of infants has been studied extensively by the school headed by the late Arild Hansen [3, 4 8] They found that the pattern of polyunsaturated fatty acids varied in a predictable manner with the content of linoleate in the diet An interpretation of their data using the methods developed in animal experimentation [1 2, 5] led to the estimate of the linoleate requirement of infants and to methods of estimating linoleate intake from analysis of serum lipid fatty acids [6] The minimum requirement of linoleate was found to be about 1 4% of total calories. The estimation of linoleate intake is based on the proportionality of $\log_1$ of the linoleate intake and the sum (diene + triene + tetraene) of the serum lipids.

In the present investigation the effects of time and of dietary fat upon the triene tetraene ratio and upon the sum (diene + triene + tetraene) of serum of infants have been studied. Comparisons were made between breast milk, cow's milk and a filled milk preparation with respect to meeting the linoleate requirement.

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[2] The Hormel Institute, University of Minnesota, Austin, Minnesota, U.S.A.

[3] Barnkliniken, Karolinska Sjukhuset Stockholm 60, Sweden.

[4] Lasarettet Skellefteå, Sweden.

TABLE 1 *Serum polyunsaturated acids in infants fed breast milk, cow's milk or a milk containing vegetable oil*

Diet	Sex	Age (days)	Polyunsaturated acids (mg/100 ml) Di	Tri	Tetra
None	M	0	10.4	3.3	5.8
	M	0	8.9	1 1	5.5
	M	0	7 6	2.2	5.7
	F	0	12.0	3.4	8.6
	F	0	14 7	2.8	8.3
	F	0	8.0	1.3	4.8
	M	0	[illegible]	4.4	8.5
	F	0	10.1	2.1	7 1
	F	1.5	11 4	2.6	9 6
Breast milk	M	0.5	8.0	[illegible]	11 8
	F	1.5	15.1	2.6	12.6
	M	[illegible]	10.3	4.8	[illegible]
	M	2.0	26.6	7 7	18.2
	M	2.0	4.4	15.0	1 7
	F	[illegible]	10.2	4.6	14 7
	F	[illegible]	10.4	4.5	7 9
	F	3.0	14 1	4 1	15.9
	F	3.5	10.7	3.2	11.9
	F	3.5	1 0	5.1	11.9
	M	4.5	4.3	1 4	12.1
	M	5.5	17.5	5.7	22.8
	F	10	[illegible]	4 1	[illegible]
	F	11	8.4	3.7	11 1
	M	1	37 1	4.1	10.5
	F	13	29.8	3.4	10.8
	M	13	57 1	8.4	13.2
	M	15	60.3	11 4	6.0
	F	16	48.3	4.3	[illegible]
	F	16.5	26.0	3.4	8.8
Breast milk	F	17	23 4	3 2	[illegible]
	F	18	22 0	2.7	[illegible]
	F	[illegible]	[illegible]	[illegible]	[illegible]
	M	123	46.0	4 3	[illegible]
Cow milk	M	8	16.7	6 9	[illegible]
	M	15	17.6	16 3	[illegible]
	M	23	26.7	7.4	[illegible]
	F	23	12.7	[illegible]	[illegible]
	F	[illegible]	13.1	8.1	[illegible]
	M	[illegible]	[illegible]	[illegible]	[illegible]
	F	40	[illegible]	3 3	[illegible]
	F	40	1	[illegible]	[illegible]
	F	41	23 8	19 2	[illegible]
	M	56	12.4	12 0	[illegible]
	M	57	[illegible]	31.0	[illegible]
	M	87	23 5	12.6	[illegible]
	M	106	14 3	[illegible]	[illegible]
Filled milk	F	—	12.2	2.3	[illegible]
	F	28	22.1	3.3	[illegible]
	F	31	41.0	3 7	[illegible]
	F	33	47.3	3 6	[illegible]
	F	36	52.0	3 8	[illegible]
	M	36	40 6	3 0	[illegible]
	M	46	33.0	3 7	[illegible]
	F	57	43 2	11 0	[illegible]
	F	59	[illegible]	3 0	[illegible]
	M	62	61.3	2.1	[illegible]
	F	63	43 6	3 7	[illegible]
	M	71	57 6	4.1	[illegible]

Materials and Methods

The infants were from the pediatric [illegible] in Stockholm and Skellefteå Sweden Blood w[illegible] btained by venous puncture at least 4 hours after the previous feeding Serum was [illegible]llected by centrifuging [illegible]nd sent by [illegible] ma[illegible] n sealed amp[illegible]ules to Austin, Minnesot[illegible] f[illegible] analyses. T[illegible] fant[illegible] were either breast fed by their [illegible] or fed formulas. The c[illegible]w milk f[illegible] [illegible]ted of pasteuriz[illegible] cows milk [illegible] with water and cont[illegible]ning 5% [illegible] [illegible] added suc[illegible]rose A filled milk cont[illegible] [illegible] and cottonseed oil as the [illegible] t and skim milk powder was given to [illegible]. This formula provided about 0. [illegible] acid by calories.

A water miscible [illegible]tamin A and D [illegible]paration was given to all infants after the age of [illegible] weeks. Some of the older [illegible] fed infants received orange juice [illegible] other fat free source of vitamin [illegible] solids or cereals were given. All infan[illegible] healthy and in good condition, except [illegible] child with a m[illegible] [illegible] and [illegible] growing hydrocephalus. His nutritional [illegible]tus was good, however. The [illegible] t[illegible]ken at various times after birth In [illegible] instances, only one sample [illegible]

infant. In a few cases, serial samples were taken from the same infant. Lipids were extracted from the serum and the component fatty acids were analyzed by alkaline isomerization [7]. The results are expressed as mg/100 ml serum.

Results and Discussion

The diets, duration of the diet feedings and fatty acid compositions of the serum lipids of the infants are given in Table 1. From these data it is apparent that the polyunsaturated acid content of serum at birth is lower than after the infant begins to take breast milk. The greatest change is found in the increasing content of dienoic acid (linoleic acid) as the infant grows older. However, in the infants fed cow's milk formula the content of polyunsaturated acids in serum does not rise with age. When filled milk containing vegetable oil is fed, the content of dienoic acid in serum rises with age in a manner comparable to that in breast-fed infants, despite the low linoleate content of the formula. Perhaps the low linoleate content is spared by the high content of short-chain fatty acids present in the coconut fat which is the principal component of the dietary fat. The chief difference between the latter two groups lies in the higher arachidonate content of the serum of the breast-fed infants.

One index of essential fatty acid status is the triene tetraene ratio. The ratios for serum fatty acids of each infant are shown in Fig. 1. The triene tetraene ratios of the newborn infant are low generally below 0.5. The ratio remains low as the duration of breast feeding increases. The ratios for infants fed cow's milk however begin to rise shortly after this

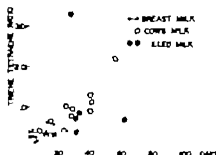


Fig. 1. Triene tetraene ratios of serum lipids of infants fed breast milk, filled milk or cow's milk as a function of time measured from birth. The dotted line indicates probable curve through the experimental points for infants fed breast milk.

diet is started. The triene tetraene ratio of serum fatty acids of infants fed filled milk formula likewise rose with time. This was caused principally by an arachidonate content of the serum fatty acids of the filled milk fed infants generally lower than that of breast-fed infants. The triene content of the two groups appears to be the same.

The expression relating dietary linoleate to tissue polyunsaturated acids [6] states a proportionality between the logarithm of linoleate intake and the algebraic sum (diene – triene + tetraene). Thus, this sum has empirical meaning as a measure of dietary linoleate. It also has biochemical meaning for the diene in tissue is largely linoleate itself and its metabolite arachidonate is the dominant tetraene. The triene is largely 5,8,11-eicosatrienoic acid which appears significantly only in deficiency and is inversely related to the essential fatty acid status of the individual. When the diene – triene + tetraene of infant serum is considered, the difference between mother's milk and cow's milk as a source of essential fatty acids

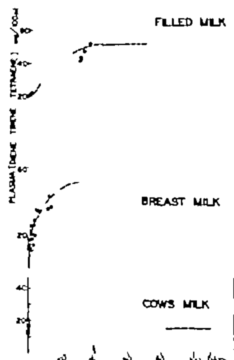


Fig — Relationship between diene – triene + tetraene of serum fatty acids and time measured from birth for three groups of infant fed breast milk, cow milk or filled milk formula. The dotted lines indicate the probable curves through the experimental points.

clearly reflected. Fig 2 shows the plot of this sum versus time on each diet The values at zero time are the same observations in all three parts of the chart When infants were fed filled milk, or breast milk the initially low values for diene – triene + tetraene rose with time whereas when infants were fed cow s milk they did not

The results presented here are in essential confirmation of the findings of Hansen and his co-workers [3 4 8], and of Woodruff et al [9] who found the level of polyunsaturated acids in serum reflects the linoleate content of the diet The present study was intended to reveal how the pattern of polyunsaturates changes with time in infants fed three diets. Using breast feeding as a standard of comparison, cow s milk formula does not induce the development of a normal pattern of polyunsaturated acids. In two infants, in the present study the serum was analyzed at different times after the feeding of cow s milk formula had been initiated In one infant whose serum was analyzed at 23 87 and 106 days the diene – triene + tetraene was found to be 47 6 ‗1.9 and 13.4 mg/100 ml, respectively The comparable triene tetraene ratios were 0 41 1 15 and 1 18 respectively In the other infant whose serum was analyzed at 8 15 20 and 57 days the diene – triene + tetraene was 26 1 ?0.8 23 7 and 35.2, and the triene tetraene ratio was 0 4 0 65 0.95 and 1 1? respectively Thus, the same relationships shown in Fig 1 and 2 for populations fed cow s milk were discernable in individuals. That is when the infants were fed cow s milk, the diene – triene + tetraene did not increase and the triene tetraene ratios rose These observations suggest that infants maintained on cow s milk formula alone do not receive sufficient essential fatty acid for normal biochemical development

Summary

The validity of the triene tetraene ratio of tissue lipids as an index of essential fatty acid deficiency and of the sum serum lipid (diene – triene + tetraene) as a measure of linoleate intake has been demonstrated previously with animal and infants. These parameters were measured as a function of time in groups of infants fed breast milk cows milk formula and a filled milk formula

Serum samples were taken from infants kept on one of these three diets for measured periods of time.

The lipids were extracted from the serum of the subjects, and the dienoic trienoic and tetraenoic acids were determined in the lipids by alkaline isomerization.

Infants fed breast milk showed low (normal) triene tetraene ratios whereas those fed cow's milk or filled milk (0.7 % linoleate calories) showed triene tetraene ratios in the serum lipids which increased with time, indicating insufficient dietary linoleate.

The sum (diene triene tetraene) increased with time in those infants fed breast milk. This appeared to be true also for those fed filled milk but the values from those fed cow's milk did not increase. These observations were also made in individuals from whom serial samples were taken.

Acknowledgement

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(L. E.) Department of [illegible]
Centrallasarettet
[illegible]
Sweden

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From the Institute for Medical Genetics, Uppsala, Sweden

Variations in Serum Leucine Aminopeptidase in Pregnant Women and in Newborn Children

by L. BECKMAN and M. GRIVEA

Serum alkaline phosphatase variants occurring only in the sera of pregnant women have been described by means of starch gel electrophoresis [6]. At the termination of pregnancy all women show these "pregnancy enzymes". There was an agreement between the alkaline phosphatase patterns found in the serum and in extract of the placentae suggesting that the pregnancy enzymes were of placental origin. Significant racial differences were found in the frequencies of the different pregnancy bands. Newborn children have a [illegible] type of alkaline phosphatase different from both the normal serum alkaline phosphatase and the pregnancy bands.

Pregnancy variations have been observed also in leucine aminopeptidase (LAP) [8, 9]. The pregnancy enzyme bands also show cystine aminopeptidase activity and have the ability to inactivate oxytocin [8].

In this paper LAP variations in late pregnancy and in new born children will be reported.

Material and Methods

At the maternity clinic, University Hospital, Uppsala, blood specimens were collected from mothers and their children at the delivery and one week thereafter. Blood samples were also obtained from a number of the fathers. Studies of the variations in this material of haptoglobin [[illegible]] and alkaline phosphatase [3] have previously been reported.

The sera were examined by means of starch gel electrophoresis in a discontinuous buffer system [1]. Staining of the gels was performed in 0.1 *M* Tris-maleate buffer pH 6.0 for 40 min at 37°C using *L*-leucyl-*β*-naphthylamide HCl as substrate and Black K salt as a diazonium dye coupler.

Results

In a sample of 145 fathers only one enzyme zone was seen migrating on the starch gel with a mobility intermediate between the albumin and transferrin zones.

In the sera of 234 mothers there were at the delivery three additional LAP zones of slower mobility (Fig. 1 and 2). They correspond to the pregnancy (oxytocinase) enzymes described by Page *et al.* [8]. Two of these zones are rather weak and therefore difficult to visualize on a photograph. In [illegible] out of [illegible]45 children an additional zone of LAP activity was observed (Fig. 1 and 2). This zone was rather diffuse and of slower mobility than the pregnancy bands.

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Fig 1

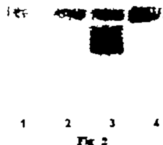


Fig 2

Fig 1 Schematic picture of starch gel showing the electrophoretic patterns of serum leucine aminopeptidase (LAP) in normal adults (2), pregnancy (3) and newborns (4). The mobilities of albumin, transferrin and slow α_2-macroglobulin are given as comparison (1) The arrow shows the direction of migration.

Fig. _ Photograph of starch gel showing the electrophoretic patterns of LAP in normal adults (1 and 4), newborns (2) and in pregnancy (3).

Already after one week the concentration of the pregnancy bands was weaker and in 154 out of 234 mothers pregnancy bands would not be distinguished with certainty in the second sample Also the band seen in the newborns showed a tendency towards a disappearance after one week, in that 16 out of 27 hildren were missing this zone in the second serum sample

Discussion

The results concerning the serum LAP show some similarities with the findings for alkaline phosphatase In both cases there exist multiple molecular forms of serum enzymes with particular enzyme variants occurring in pregnancy and others in newborn children

All newborn children have the childhood alkaline phosphatase but only about 10% of these children have the childhood variant of LAP The explanation of this phenomenon could be that the childhood LAP is a foetal enzyme and that most individuals have ceased to synthesize it at birth. A pronounced variability in the onset and cessation of protein synthesis in the perinatal period is well documented. Foetal haemoglobin is e g regularly present at birth and normally it disappears rather soon except in cases of hereditary persistence of foetal haemoglobin [7]. A reversed picture is shown by haptoglobin, which is present in only about *0 % of newborn children. After some months almost all individuals have started to synthesize haptoglobin except for some few cases of hereditary ahaptoglobinaemia [ ].

Genetic variations in LAP isozymes have been found both in animal [4] and plant material [5] but polymorphism has so far not been demonstrated in the human serum LAP enzymes. The origin and the physiological significance of the LAP pregnancy enzymes are questions that remain to be investigated.

Summary

Electrophoretic variations in serum leucine aminopeptidase (LAP) were investigated in adult males pregnant women and newborns. The males have in their sera only one zone of LAP activity Pregnant women show at delivery three additional LAP zones These pregnancy enzymes tend to be weaker or disappear already after one week. About 10 % of the newborns show in the cord sera a childhood variant of LAP

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The Institut for Medical Genetics
V Ågatan 24
Uppsala
Sweden

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From the Department of Virus Research Karolı Institutet, School ... the Pediatric Clinic and Bacteriological Laboratory Ka ka Sjukhuset, St Sweden

Measles Vaccination

III Serologic Responses to Immunization with Purified Hemagglutinin

by E. NORRBY R. LAGERCRANTZ, S. GARD and GUN CARLSTRÖM

Measles virus contains two main antigenically active structures, the hemagglutinin (HA) and the nucleoprotein, which can be separated by treatment with Tween 80 and ether [5 6, 10]. This mild treatment does not destroy the immunogenic activity of the HA [10 Norrby unpublished]. Accumulated indirect evidence indicates that the HA is the virus antigen responsible for the stimulation to production of neutralizing antibodies [7]. Attempts were therefore made to isolate this antigen in a relatively pure stat from a Tween 80 and ether treated material and to test the immunogenic effect of such a product in human beings. For comparison a group of children immunized with the formalin-killed vaccine previously studied [4 8] was also included in the field trial.

Material and methods

Vaccines

The purified HA was prepared as follows: A subline of the Edmonston strain of measles virus was propagated in primary dog kidney cells maintained on Earle balanced salt solution with 0.5 % lactalbumin hydrolysat and % calf serum. The final change of medium was made when the characteristic cytopathic effect started to appear. After incubation at 37°C for a further t 10 days to allow accumulation of antigen the material was harvested by three times freezing and thawing. The presence of satisfactory HA activity in individual bottles was controlled and the pooled material treated with Tween 80 and anesthetic ether as previously described [3]. Remaining ether was removed by evacuation. A small amount of octyl alcohol was added to prevent extensive foaming. After volume reduction by forced dialysis the material was purified by filtration on Sephadex G-200 and equilibrium centrifugation in CsCl gradients [5]. Less than 1 % of the original amount of protein was present in the final product which was diluted in phosphate buffered physiological saline pH 7.2 (PBS), t a volume corresponding to 1 5 of the pooled starting material. The titer of the vaccine, hereafter called the TE vaccine was 4000 to 8000 HA units per ml. Before being used the vaccine was submitted t the relevant safety tests prescribed for the Swedish polio virus vaccine.

The second type of inactivated vaccine used was kindly provided by Chas. Pfizer & Co., Inc. It was prepared from formalin killed (FK) monkey kidney tissue culture grown material by precipitation together with aluminium phosphat In the text

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below the vaccine will be denoted FK vaccine

Attempts to compare the potency of the two vaccines by intraperitoneal inoculation of one ml of serial three fold dilutions into groups of 5 guinea pigs indicated the TE vaccine to have an antigenic extinction limit t ter 3 to 4 times higher than the FK vaccine.

Study population and vaccination procedure

Normal and healthy children, aged 6 to 4 months, without detectable pre immunization antibodies were selected for vaccination. The children were preferably selected from families with one more child without known exposure to measles.

Three monthly doses of 1 ml FK or TE vaccine were given intramuscularly and 0.2 ml whole blood was collected in 1.2 ml of tissue culture medium containing heparin 1 50 000 at each time of injection and in addition 7 to 10 days and 11 months after the last injection. After removal of the erythrocytes the samples, which were considered to represent a serum dilution of 1:10, were inactivated for 30 minutes at 56°C before being used in the tests.

Serological analyses

Neutralization (NT), hemagglutination [illegible]bition (HI) and complement fixation [illegible] tests were performed as previously described [4, 6]

Results

Side reactions

No local or general reactions were observed with the TE vaccine Nodular indurations at the places of the three injections of FK vaccine were fairly common and in two out of 40 children they developed into sterile abscesses In both cases incisions of the abscesses were made

Serologic responses to vaccination

The number of children without pre-immunization antibodies, who received

TABLE 1 *Frequency of conversions and geometric mean titers after immunization with two different vaccine products.*

The sera were collected 7 to 10 days after the last dose of vaccine Lowest dilution tested: NT 1 [illegible]0, HI 1:40 and CF 1 [illegible]0 or in [illegible] few sera 1 [illegible].

Vaccine	Serologic test	Frequency of conversion	Geometric mean titers in positive sera
TE	NT	37/37[a] 100 %	1 1[illegible]0
	HI	40/40 100%	1 [illegible]70
	CF	13/40 –32.5%	1 [illegible]
FK	NT	[illegible]8/28[a]–100 %	1 1[illegible]0
	HI	31/31 100%	1:610
	CF	5/31 –80.6%	1 37

[a] Three sera per vaccine were lost due to fungal contamination of the neutralization test.

three doses of vaccine and who were not exposed to measles during the course of vaccination amounted to 40 and 31 with the TE and FK vaccine respectively Frequency of conversions and geometrical mean titers as determined by the three serological methods used are given in Table 1 The two vaccines gave 100% conversions and the mean NT and HI antibody titers were of similar order of magnitude In the CF test the frequency of conversions was considerably higher among children given the FK than in those receiving the TE vaccine 80.6 as compared to 32.5% on a serum concentration level of 1 [illegible]0

The development of HI serum titers during the course of vaccination was followed by analysis of sera collected at the time of injection of the second and the third dose of vaccine in addition to the post vaccination serum sample Fig 1 illustrates the results of these tests in the form of immunity profiles of the two groups of children. The vaccines behaved

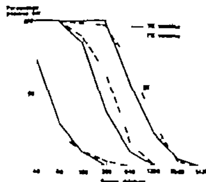


Fig. 1 The HI "profiles of groups of children vaccinated with the TE (—) and FK (-) vaccine The curves demonstrate from left t right profiles one month after the first (I), one month after the second (II) and 7 to 10 days after the third (III) of the monthly inoculations.

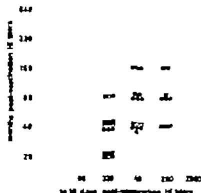


Fig. 2. Correlation between early and late post vaccination HI titers. Sera from children im munized with TE (□) and FK (●) vaccine (▲) represents sera from child given the latter vaccine who contracted measles-like disease during the time of observation.

almost identical in spite of the fact that potency tests suggested the TE vaccine to contain more virus-specific antigen than FK vaccine A slight difference in profiles is suggested in the one month samples. The reason for this might be that the FK vaccine contains antigen adsorbed to alum, whereas the TE antigen is suspended in phosphate-buffered physiological saline without addition of adjuvant

Persistence of antibodies over a period of 11 month fter vaccination

A comparison of HI serum titers in samples collected 7 to 10 days and 11 months after the last dose of vaccine is given in Fig and of immunity profiles on these two occasions in Fig 3 The number of children vailable for follow up studies were 36 out of 40 and 22 out of 31 for the TE and FK vaccine respectively One child (M L) who reacted with mild clinical symptoms upon exposure during the time of observation (see below) was excluded in Fig 3 In the late post vaccination serum samples antibodies were still detectable among 100% of children given either one of the two vaccines. Fig 2 shows that there is a good correlation between serum titers in early and late post vaccination sera. The only two exceptional sera are one from the measles-exposed child mentioned above and the other from a child with relatively low early post vaccination titer of 1 70 This latter child actually exhibited a rise in titer to 1 160 although no known exposure had occurred. As can be seen from Fig 3 the magnitude of displacement of the profiles" toward lower titers is similar for the tw vaccines. The reduction in geometric mean serum titers were 10 6 times with the TE vaccine and 8.5 with the FK vaccine. Statistical analysis revealed the slight difference between the two values to be without significance

The effect of nat ral xposure to measles

Comparatively few cases of measles occurred in the community during the 11

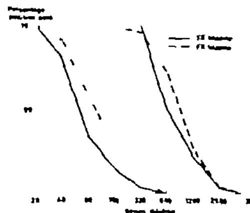


Fig. 3. Comparison of the HI "profiles" at two different times in groups of children immunized with TE (—) and FK (- - -) vaccine. Serum samples collected 7 to 10 days—right "profiles"—and 11 months—left "profiles"—after vaccination were analyzed.

months of observation that at the time of the present presentation has elapsed after vaccination. No clear-cut exposures were registered among the vaccinees. In spite of this, one child—M. L.—who had received FK vaccine developed a measles-like rash and exhibited a rise in temperature to 39.5°C for 2 days 6 months after vaccination. She was very slightly affected by this condition. The HI serum titer was 1:640 both in the 7 to 10 day and 11 months postvaccination sample which makes it likely that the child actually had contracted a mild measles infection.

Discussion

The aim of the present investigation was to make a comparative analysis of the immunizing effect of purified measles HA (TE vaccine) and a formalinized, alum adsorbed, whole virus vaccine (FK vaccine). The serologic responses in the group of children given the latter vaccine corresponded well to results previously obtained with the same product [4]. Geometric mean serum titers in NT and CF tests were almost identical, whereas the mean HI serum titer was somewhat lower in the field trial described here 1:890 as compared to 1:1500. This probably reflects a small variation in the HI antigens applied in the tests. However reference sera were not included in the tests. The decrease in antibody titers with time after immunization also is in good agreement in the previous [8] and the present field trial. The reduction was 6- to 7-fold over a period of 8 to 9 months in the former case and 8- to 10-fold during 11 months in the latter case.

Although the antigenic extinction limit titer in guinea pigs of the TE vaccine was 3 to 4 times higher than the corresponding titer of the FK vaccine no significant difference in appearance of neutralization and HI antibodies were detectable among the vaccinees. In analogy with this it was found in a previous field trial [4] that immunization with 0.5 or 1.0 ml vaccine doses did not affect the serum titers reached. It has furthermore been described by Peck [9] that increase of the amount of antigen administered up to a certain level increased the mean serum titers among groups of vaccinees. However no further increase was obtained above this dose level. It would seem therefore that once an inactivated vaccine of a certain critical potency is used the major factors determining the final level of immunity are the number and probably the spacing of injections. This problem deserves further systematic studies.

A marked difference was found in geometric mean CF titers between the groups of children given the two vaccines. This

might be attributable to the presence of antigens associated with, for example, the internal component or the lipids of the viral envelope which presumably are present in a higher concentration in the FK than the TE vaccine The working hypothesis behind the development of the TE vaccine is that antibodies interacting with these antigens, which are without importance for neutralization in vitro do not have any protective function in vivo either This hypothesis can only be tested by analysis of the long time protective effect of immunization with purified HA in human beings

The various advantages of using a purified inactivated measles antigen for immunization against measles have been discussed in other connections [1 6] Some more obvious in their nature are the filtrability of the vaccine, the absence of non-essential antigens interfering with the antibody response to the specific antigen and the suitability for incorporation in a polyvalent vaccine. Among others of still hypothetical importance can be mentioned the absence of the main part of the viral lipids which have been suggested to play a role in the postulated hyperergic reactions in measles encephalitis [2]

It should be pointed out that the methods here used on an experimental scale for production of the purified HA are not suited for large scale manufacturing The problem of finding a simplified preparatory procedure is at present under investigation.

Summary

A comparative study was made of the immunizing effect of purified measles hemagglutinin (TE vaccine) and a formalinized, alum-adsorbed, whole virus preparation (FK vaccine) Three monthly doses of 1 0 ml vaccine were given to two groups comprising 40 and 31 children, respectively Both vaccines gave 100 % conversions with almost identical mean neutralization (about 1 180) and HI serum (about 1 600) titers. A difference was found in the CF tests The frequency of CF conversions was 80 6 % with the FK vaccine and only 32.5 % with the TE vaccine

Blood samples collected 11 months after the administration of the last dose of vaccine were submitted to serological analyses. HI antibodies were still present on a detectable level in 100 % of the sera. The reduction in mean titers was 8.5 and 10 6 fold in groups of children given the FK and TE vaccine respectively No known exposure to measles occurred during the time of observation. However 6 months after vaccination one child given the FK vaccine contracted what most likely was mild measles.

Acknowledgements

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(E. N) Department of Virus Research
Karolinska Institutet Box 764
Stockholm 1
Sweden

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From the Department of Medicine and Premature Infant Center [1] University of Colorado School of Medicine Denver Colorado, U.S.A

Fibrinolytic Activity Thrombin Inhibitor and Kinetics of Clot Formation in Premature Infants with Respiratory Distress Syndrome

by KURT N VON KAULLA, EDITH VON KAULLA and JOSEPH BUTTERFIELD

Combined studies of fibrinolytic activity thrombin inhibitor and the kinetics of clot formation in premature infants with the respiratory distress syndrome have not been carried out before Information on each of these phases of clotting is very scanty in healthy premature infants. Although combined studies of these phases of clotting have not been done in full term infants either all three have frequently been found individually to attain quite different levels from adult ones. Since at least two of them (fibrinolytic activity thrombin inhibitor) represent an increased activity rather than a reduction of components of the clotting and fibrinolytic system, it was of interest to study their activity in premature infants with the respiratory distress syndrome (RDS)

Material and Methods

Fourteen premature infants with RDS and varying degrees of acidosis were studied during the first 14 hours of life. The diagnosis of RDS was a clinical one based on persistence of grunting respiration, retractions, and usually cyanosis during the first six hours of life

Laboratory procedures

( ) Drawing and processing of blood Four ml blood were drawn through a short plastic catheter from the umbilical vein into a 5 ml syringe which contained 1 ml 3.8 % sodium citrat U.S.P Blood and citrat were gently mixed in the syringe and transferred into a centrifuge tube and spun 5 minutes at 1400 g. The plasma of six children was deep-frozen for a few hours before testing This procedure does not affect the thrombin inhibitor [10], has a slight reducing effect on the fibrinolytic activity [12] and tends to speed up the onset of fibrin formation in the Thrombelastograph. However the observed deviations from normal adult values were still so gross that the frozen samples were included in the studies.

(*b*) Thrombelastograms: The Thrombelastograph [8, 9] was used to record the kinetics of clot formation.

( ) Thrombin inhibitor: The activity of thrombin inhibitor was determined as the thrombin time of plasma without and with addition of calcium chloride [10]

(*d*) Fibrinolytic activity: The fibrinolytic activity was estimated primarily as plasminogen activator by the euglobulin lysis time [11].

( ) Fibrinogen: Fibrinogen was determined as fibrin with a conventional method [13]

(*f*) pH determination The Astrup Radiometer using umbilical vein blood.

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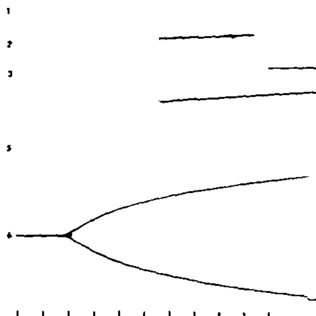


Fig 1 Thrombelastograms of fresh recalcified citrated plasma of premature infants with respiratory distress syndrome. Infants 1 (1400 g), (1600 g) and 5 (925 g) died. Infants 3 (1100 g) and 4 (620 g) survived. Normal infant (6) was born at term. Time scale: 2 and 10 minutes intervals.

Results

The data with euglobulin lysis time and thrombin time are compiled in Table 1 Representative examples of the kinetics of clot formation in fresh plasma as measured with the Thrombelastograph are shown in Fig 1

Euglobulin lysis time. There was a very marked shortening of the euglobulin lysis time in twelve of thirteen premature infants This shortening was present in infants studied soon after birth as well as in those studied up to 14 hours later The average value amounted to 4* minutes. This is about one third of the lower normal value for adults, which is above 1°0 minutes. There was no relationship between euglobulin lysis time and the pH of the blood.

Thrombin time. The thrombin time was considerably prolonged in each of the 14 premature infants with RDS. The average value was 37 seconds (adult normal, 17 seconds) Both euglobulin lysis time and thrombin time can be influenced by the fibrinogen level. However only very low fibrinogen levels would tend to shorten the euglobulin lysis time or to prolong the thrombin time [7] There was furthermore the theoretical possibility that the fibrinogen would have a reduced reactability to thrombin thus producing a prolonged thrombin time The presence of calcium chloride reduces the activity of thrombin inhibitor to a great extent [17]. Therefore a marked shortening of the prolonged thrombin time upon addition of calcium chloride would prove a normal reactability of the plasma fibrinogen to thrombin. Such shortening did indeed occur as shown

TABLE 1 *Euglobulin lysis time and thrombin times together with birth weight time of sampling of blood after birth, pH of blood and causes of death in 14 premature infants with RDS*

Patient	Age (hours)	Birth weight (g)	U V pH	Euglobulin lysis time (minutes) Normal, > 1?0	Thrombin time (seconds) Normal: < 1	Remarks
You.	0.5	870	7 10	19	43	4*
Lun.	2	1400	7..1	28	39	1 died
Lyo.	2	1830	7 16	18	40	
Bil.	2	2290	7.21	48	46	
Mil.	2	1820	—	40	33	
Had.	3	1290	7.26	18	44	
Mar	3	1800	7 19	26	28	died
Gul.	5	925	6.70	35	63	5 died
Dalt.	5	1060	6.64	—	29	died
Son.	5	1000	7 09	60	38	.. died
Ren.	6	1100	7.00	37	33	3.
Tur	9	1474	7 18	190	.5	
Pol.	9	2060	—	60	29	
Wil.	14	1530	7.24	46	29	
Average				42	37	

Causes of death.
Lun.: Atelectasis, hyaline membranes, subarachnoid hem.
Mar Atelectasis, hem. falx cerebri and tentorium.
Gul: Atelectasis, hyaline membranes.
Dalt.: Atelectasis, hyaline membranes, intraventr and subarachnoid hem.
Son.: CNS. hem.

You. Expired at 100 days of age with pneumonia.

in Table Table 2 indicates clearly that there is (a) no markedly lowered fibrinogen level, and that (b) the thrombin time was shortened to more than half of its original value after addition of calcium chloride Consequently there was no technical interference with the test results. The short euglobulin lysis time reflects therefore a pronounced increase in the titer of the plasma plasminogen activator and the prolonged thrombin time i caused by a real and marked increase in the titer of thrombin inhibitor in the plasma of premature inf nts with RDS.

Kinetics of clot formation (thrombelastograms) In all premature infants studied there were t times, grossly abnormal kinetics of clot formation. The thrombelastograms showed that the fibrin formation proceeds once it has started (splitting of black line) very slowly (flat slope of diverging lines) producing a structurally bnormal clot (thrombelastograms 1 .. and 3) which resembles the ones obtained from severe thrombocytopenic adult pa tients. (Very flat slope of diverging lines; black center strip.) The beginning of measurable fibrin formation is in some cases delayed (thrombelastograms 3 and 4) However such delay is not present in thrombelastograms 1 and 5. Here the fibrin formation starts much earlier after recalcification and at such times which would indicate a tendency to hypercoagu

TABLE 2 *Combined studies of euglobulin lysis time and fibrinogen content and corresponding thrombin times in absence and in presence of calcium chloride respectively in four premature infants with respiratory distress syndrome*

Euglobulin lysis time (minutes)	Fibrinogen mg%	Thrombin time (seconds) Without $CaCl_2$	With $CaCl_2$
25	250	63	4
46	[illegible]84	[illegible]9	13
18	188	44	[illegible]0
18	2[illegible]4	40	19

lability (premature clot formation) in adults. Six additional thrombelastograms were obtained from frozen plasma. They show without exception early or very early beginning of fibrin formation, this is also true for previously frozen adult plasma (due to the activation of clotting factors, breaking up of the platelets). These observations with frozen plasma of premature infants with RDS indicate that their plasma behaves essentially the same as adult plasma in terms of initiation of fibrin formation after freezing.

Discussion

To discuss blood coagulation or fibrinolytic activity in newborn infants is to enter a field of controversy. The reduction of various clotting factors at this age has been documented by many investigators. However, there are also numerous reports (for older literature see [3]) indicating that the blood of newborn infants clots fast and that they exhibit hypercoagulability, particularly in the blood obtained from the umbilical vein. Hypercoagulability in newborn infants has recently been pointed out again [15, 16].

A similar controversial situation prevails in respect to fibrinolytic enzymes in newborn infants. There is agreement that proactivator and plasminogen are reduced (there is less agreement in the case of antiplasmin) in prematures and in infants at term. And yet there are many observations of a marked increase of fibrinolytic activity (for literature see [1*]). The fibrinolytic activity has been reported to disappear within three days after birth [4] and by other investigators within one hour after birth [6]. Interestingly enough, increased fibrinolytic activity has been found by all investigators, using blood from the umbilical vein, the umbilical artery, the iliac artery and the iliac vein. It has been stated that the fibrinolytic activity is more pronounced in premature or stillborn infants as compared to infants at term [ ]. The highest fibrinolytic activity was reported in a small embryo (nine weeks of gestation); it diminishes with increasing age [**]. A normally functioning liver clears plasminogen activator from the circulating blood [13]. Therefore it might be possible that the fetal liver slowly acquires this clearing ability with increasing age. The respiratory distress syndrome in premature infants does not, as indicated by the presented data, affect the marked increase of plasminogen activator activity of the circulating blood during the first fourteen hours after birth.

Together with the increased plasminogen activator activity there was an equally marked increase of thrombin inhibitor. Increased thrombin inhibitor activity has been found in infants born at term (but reduction has also been seen, for discus-

sion see [3]) and in premature infants [?0] The premature infants with respiratory distress in this report exhibited a marked increase of thrombin inhibitor The source and mechanism of the increased thrombin inhibitor are not known. In adults, a rise of thrombin inhibitor can be observed in conjunction with circulatory dysfunction [14]. The observed rise of thrombin inhibitor in these premature infants with RDS however marked, was in general not of such nature that an effect on the coagulation process could be expected. The test was carried out in citrated plasma Citrate enhances the inhibitor activity Ionized calcium as it is present in whole blood, diminishes its activity considerably Higher inhibitor levels than the ones observed would tend to effect th coagulation process if observations in adult are valid for comparison.

The kinetics of clot formation in the recalcified plasma of this series of premature infants was, as demonstrated by the thrombelastogram definitely defective when compared with dult plasma. However in several instances the beginning of clot formation was normal if not accelerated. This occurred in infants who died with the respiratory distress syndrom (thrombelast grams 1 2 and 5 in Fig 1) Such observation then would tend to confirm the hypercoagulability of the blood from the umbilical vein observed in prematures at the seventh month of gestation [1] or with the thrombelastogram in infant born t term [1]. A tendency to hypercoagulability was lso observed with the thrombelastogram in prematures without RDS during the first ten days of life other thrombelast grams of the reported series were normal [10] Besides the earlier onset of clotting as observed in our series of premature infants the structure of the clot on the other hand, was quite defective as compared with clots obtained from adult plasma. Whether or not such comparison is permissible must remain subject to further studies The presence of a fetal fibrinogen, different from the adult and more rapidly transformed into fibrin by thrombin, has been claimed [15, 16]. The comparison in our study was based on citrated plasma and results obtained with whole blood might be different. Thrombelastographic tracings of whole blood are less sensitive in many respects for clotting deviations than the ones obtained with recalcified citrated plasma. The results with the studies of the kinetics of the clot formation in premature infants with RDS are ambiguous indicating that an early begin of the clotting process may occur but that a structurally abnormal clot is produced in all instances. As compared with adults, the structural abnormality would indicate a deficient platelet function. Suggestive evidence of such abnormal platelet function in the presence of a normal thrombelastogram has been claimed by one investigator of premature infants [19] Abnormal thrombelastograms similar to ours and indicative of abnormal plat let function have been presented by others for the first ten days of life in infants born t the seventh and eighth month of gest tion [ ]. There is general greement that the platelet count in prematures, while subject to marked variations, is normal. The question of balance between hyper and hypocoagulability in th blood of prematures (and in infant born t term) call for further studies. Thi is particularly impor

tant in view of the known interaction of the increased fibrinolytic activity with the activation of the clotting system (for literature see [12]).

Summary

Marked increase of plasma plasminogen activator activity was found together with increased thrombin inhibitor activity in premature infants with the respiratory distress syndrome. Simultaneously obtained thrombelastograms may indicate either a delay or an early begin of clot formation. A structurally abnormal clot was obtained in all instances.

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University of Colorado
Medical Center
4200 East Ninth Avenue
Denver
Colorado 80220
U.S.A.

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CASE REPORT

Intermittent Disappearance of the Continuous Murmur of Patent Ductus Arteriosus

by IRMA HYRSKE, B LANDTMAN L LOUHIMO and LEENA TUUTERI

From the Children Hospital, U ersity of Helsinki, Finland

Spontaneous disappearance and subsequent return of the continuous murmur of an isolated patent ductus arteriosus unassociated with pulmonary hypertension is a rare phenomenon. In 1960 Shapiro and his associates [3] made this observation in a 9-year-old girl with patent ductus arteriosus and they stated that the phenomenon had not to th ir knowledge been previously described One similar case has subsequently been reported by Keith & Sagarminaga [1].

We recently had the opportunity of studying a child with patent ductus arteriosus in whom for no apparent reason, intermittent disappearance of the continuous murmur occurred

Case Report

The patent girl 5 years and 9 months of age was admitted t the hospital for the first time t the age of 1 year for surgical correction of poly lactyly of hands and feet. Physical examination revealed, in addition to these anomalies, a continuous murmur accompanied by a thrill t the upper left sternal border The cardiac findings were considered t be characteristic of a patent ductus arteriosus Following plastic surgery of the fingers and toes, the patient was placed on the waiting list for cardiac operation.

The patient had remained asymptomatic during the intervening period. She weighed 19 kg and was 116 cm tall. Her general condition was good and the color of the skin was normal. A harsh continuous murmur accompanied by a thrill was heard at the second intercostal space to the left of the sternum. There was a faint apical gallop. The blood pressure was 120 50–0 mm Hg in the arms and 140 60–0 mm Hg in the legs. The femoral pulsation were of water hammer-type

Radiological examination of the chest showed moderate cardiac enlargement (450 ml/sqm of body surface) There was prominence of the pulmonary conus, the left ventricle and the left atrium. Th hilar vascular markings were increased.

The electrocardiogram showed a balanced axis, vertical position of the heart nd moderat left ventricular hypertrophy Phonocardiography demonstrated a typical continuous murmur with high amplitude over th pulmonic area (Fig. 1A).

Examination of other organs did not show anything abnormal Tests of blood and urine were normal.

The findings were again thought t be typical of patent ductus arteriosus and surgical correction was recommended.



Fig 1 (A). The phonocardiogram shows typical continuous murmur over the pulmonic area. (B). The murmur has temporarily disappeared for no apparent reason.

Routine auscultation in the ward the following day did not however to the surprise of everyone elicit any murmur. Several observers were present, one of whom had examined the child when she was first seen in the hospital at the age of 1 year. The patient was immediately sent to an adjoining room for phonocardiography but by the *time she arrived there the murmur and thrill* had reappeared. Various manipulations were then tried in order to cause the murmur to [illegible]ear again but without success. These [illegible]lations included exercise auscultation of the child in different positions, cough [illegible] maneuver the breathing of cold air jugular venous compression and carotid sinus massage.

The patient was carefully studied during the ensuing days. On a few occasions, the murmur was found to disappear for a few minutes for no apparent reason (Fig 1B). The disappearance and return of the murmur *usually occurred gradually within the space* of a few seconds.

Right heart catheterization was performed 8 days after admission of the patient to the hospital. The catheter entered the aorta from the pulmonary artery through a ductus, the course of which appeared normal. The pressure was 96/40 mm Hg in the aorta, 45/25 mm Hg in the pulmonary artery and 45/0 mm Hg in the right ventricle. Blood samples showed a marked rise in oxygen in the pulmonary artery but no additional left-to-right shunts. The arterial oxygen saturation was 99%. It should be mentioned that the continuous murmur persisted throughout the catheterization.

The patient was operated on 7 days after *cardiac catheterization. Following* thoracotomy no definite thrill could be felt over the ductus. The ductus was dissected free. It was 9 mm long and its diameter was 7 mm. At this stage a definite thrill was felt at the pulmonary end of the ductus. The ductus left the aorta slightly more cranially than usually but its shape and direction appeared normal. Temporary clamping of the ductus immediately abolished the thrill. Several attempts were now made to make the thrill disappear by changing the position of the heart and by applying different retractors but without result. The ductus was ligated *using 4 silk sutures*, since it was thought that division of the ductus would have involved a slightly greater risk. The postoperative course was uneventful.

Following operation, the heart sounds and phonocardiogram remained normal. The child was discharged from the hospital 2 weeks after the operation.

Discussion

It seems safe to assume that the intermittent disappearance of the continuous murmur of a patent ductus arteriosus is due to temporary arrest of the blood flow through the ductus. The cause of the arrest remained obscure in the present case. The patient had a mild pulmonary hypertension but the pressure gradient between the aorta and the pulmonary artery seemed big enough to exclude the possibility of the pressures becoming temporarily balanced thus abolishing the murmur

In the case described by Shapiro and his associates [3], there was a slight angulation of the ductus at its junction with the pulmonary artery The thrill disappeared when gentle dorsal traction was applied to the pulmonary artery during surgery It was thought that the intermittent disappearance of the murmur observed before the operation was due to temporary occlusion of the ductus caused by slight changes in the position of the mediastinum Such a mechanism seemed unlikely in the present case since the ductus appeared normal and the thrill was not caused to disappear by altering the position of the heart and the great vessels during operation.

Post mortem studies have shown the occasional occurrence of a membrane-like formation at the pulmonary end of a patent ductus arteriosus. This observation has been made in 3 cases by Wagener [6] and in _ cases by T usig [4] Keith & Sagarminaga [1] reported the case of a 10-year-old girl with patent ductus arteriosus who exhibited intermittent disappearance of the continuous murmur At surgery the ductus was divided and it was found to contain a valve or veil-like structure at its junction with the pulmonary artery Apart from this finding, the ductus appeared normal These investigators thought that the valve could have accounted for the intermittent disappearance of the murmur

Our case had many features in common with that described by Keith & Sagarminaga It is possible then, that a similar valve mechanism may have temporarily interrupted the blood flow through the ductus giving rise to the unusual auscultatory findings

Intermittent disappearance of the continuous murmur of a patent ductus arteriosus is, as in the present case a chance discovery Are these cases possibly more common than is generally believed? Could this phenomenon, for instance explain the course of events in some cases where the continuous murmur of a patent ductus arteriosus has not been heard at previous examinations?

Summary

A $5_{1/2}$-yea -old girl with patent ductus arteriosus exhibited intermittent disappearance nd return of the continuous murmur The ductus left the aorta slightly more cranially than usually but its outward appearance was normal The cause of the unusual auscultatory findings is discussed.

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Department of Cardiology
The Children's Hospital
University of Helsinki
Helsinki, Finland

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CASE REPORT

Juvenile Diabetes Mellitus Associated with Acute Pancreatitis

by JOHN S. CYWINSKI, FRANK A. WALKER, HARVEY WHITE and HOWARD S. TRAISMAN

From the Division of Endocrinology and the Department of Radiology, the Children's Memorial Hospital, Chicago, Illinois and the Departments of Pediatrics and Radiology Northwestern University Medical School.

The occurrence of acute pancreatitis in the pediatric age group is rare [1], and the association with diabetes mellitus is extremely uncommon [5]. There have been three patients with this association at The Children's Memorial Hospital since 1921. We are presenting two additional cases of acute pancreatitis and diabetes mellitus to the one previously reported from this hospital [10].

Case Reports

Case 1

L. G. a 12.5-year-old white female was transferred to The Children's Memorial Hospital from another hospital on March 6, 1961 with a two-day history of anorexia, weakness and severe epigastric pain. She had vomited once and had been afebrile.

Past history included an episode of clinical parotitis in July 1959 which was treated with antibiotics. One month later enlargement of the thyroid gland was noted, for which Lugol solution was prescribed without any laboratory investigation or follow up care. There was no history of diabetes or thyroid disease in the family.

Physical examination revealed a seriously ill, moderately dehydrated, semiconscious girl who had Kussmaul respirations. Temperature was 38.5°C, pulse 130/min., respirations 26/min, and blood pressure 126/78. She weighed 33.2 kg and was 137.5 cm tall. The skin was dry and she had a sallow complexion. Her hair was dry and of fine texture. The pupils were dilated but reacted to light; the fundi were pale. Ears and nose were normal. The mouth and throat revealed a mildly reddened pharynx and a dry coated tongue. Her breath had a strong acetone odor. The neck was supple and the thyroid gland was enlarged. Examination of the lungs, heart, extremities, genitalia and nervous system was normal. The abdomen was slightly distended and peristaltic sounds were not audible. There was tenderness in the upper abdomen, with rigidity and rebound tenderness. The liver and spleen were not palpated. Initial urinalysis showed % glycosuria and moderate acetonuria. The CO_2 was 8.1 mEq/L, sodium 120 mEq/L, chloride 89 mEq/L, serum amylase 175 units, and blood sugar 265 mg %. A white blood count the morning after admission was 12,600 with polys 31 % and bands 53 %; the hemoglobin could not be read because of hyperlipidemia.

The initial diagnosis was diabetes mellitus and possible acute pancreatitis. She received our standard treatment for diabetic acidosis [12] consisting of intravenous fluids and regular insulin. An antispasmodic, penicillin and streptomycin were given for the

TABLE 1 — *Case 1*

	Blood sugar	Serum amylase	Cholesterol	Cholesterol esters	Phospholipid	Tri-glyceride	Total plasma lipid
Normal ...	60–90 mg %	80–200 unit	150–250 mg/100 ml	150–220 mg/100 ml	160–310 mg/100 ml	0–190 mg/100 ml	570–820 mg/100 ml
1961							
March 6	263	175	1500	off curve		> 1000	
7	185						
8	186		1055		2216	54	3103
9	231						
10	91	120[a]					
12	227		425	107	660	221	1618
14	46	40					
18			330	202			
20			463	246	604	134	1470
April 25	162	66	215	150	243	215	1093
Nov 1		95	233	180	247	186	1610

[a] Peritoneal fluid

Note Reference to lipid studies no 13

five days, and then penicillin and mystecilin F were prescribed for another five days. A transfusion of 300 ml whole blood was given on March 10 because of a low hematocrit. Improvement was gradual. Six hour diabetic management was started on March 12, and on March 16 a weighed diabetic diet or her age plus lente and regular insulins were instituted. Recovery was now uneventful and she was discharged on March 25, 1961 with a daily dosage of 32 units lente and 16 units regular insulin before breakfast.

During hospitalization there was marked hyperlipidemia (Table 1). On the fifth hospital day peritoneal fluid was aspirated which contained mildly elevated amylase activity 120 units. On March 8 1961 serum calcium was 8 1 mg (normal 10–12 mg %), but it subsequently returned to normal levels.

A chest x-ray was normal. Supine upright and lateral decubitus films of the abdomen on March 7 1961 were interpreted as follows: "The transverse and ascending colon contain a moderate amount of gas, especially the transverse colon, which appears to be definitely dilated. The gas pattern goes up to the splenic flexure and abruptly ceases. The localized ileus [illegible] involving the transverse colon and also the collection of gas in the duodenal loop is highly suggestive of acute pancreatitis."

An upper gastrointestinal series on March 15 1961 was reported as follows: "The stomach reveals definite retention of fluid. The first portion of the duodenum is irregular and appears narrow. Spot films revealed gross irregularity along the margins, probably indicative of edema of the mucosa. The second and third portions of the duodenum are definitely more dilated than the first portion; these changes are compatible with acute pancreatitis." Repeat examination on March [illegible] 1 revealed improvement in the appearance of the duodenal loop, indicating favorable progress of the pancreatitis.

Table 1 summarizes the pertinent chemical findings during her three hospitalizations.

The child was readmitted to this hospital on April 24 1961, for evaluation. Her diabetes was well controlled and her weight was 34.5 kg, height 138.1 cm. Insulin dosage at the time of hospital discharge on April 27 1961 was 30 units lente and 18 units regular insulin daily. An upper gastrointestinal series and gallbladder x ray were normal. The PBI was 6.9 mcg/100 ml.

TABLE 9 — *Case 2*

	Blood sugar	Serum amylase	Serum lipase	Urine amylase
Normal	80–80 mg %	60–200 units	< 1.0 unit	6–40 units
1962				
Nov 7	540	749	1.1	
8	62	678	2.2	2034
9	72			3900
10	113	433	2.1	
12	63	414	2.1	3230
14		330	—	
15	262	160		
16	250	248	—6	7150
19		190	3.6	

Her third hospital admission was November 21 1961 for a thyroid gland biopsy At this time she weighed 47.4 kg and was 141.5 cm tall. Her skin and hair were of normal texture She was taking 46 units lent and 30 units regular insulin each morning A biopsy was performed on November 22, 1961 by Dr William L. Riker The biopsy diagnosis was chronic thyroiditis (Hashimoto struma)

This patient has had regular follow-up care and is doing well. She is good student has normal activity and is under good diabetic control. Menstruation began in April, 196_, and has been normal in amount and duration, occurring every 28 days.

On June 15, 1963, she weighed 48.2 kg and was 146 8 cm tall. Her thyroid gland was unchanged and she continued to have regular menses. Blood pressure was 110/ 0, cholesterol 140 mg%, fasting blood glucose 190 mg%, and she was taking 42 units lente and 4 units regular insulin every morning

On November 2, 1963, she weighed 49 2 kg and her height was 148.3 cm. Her diabetes was well controlled with 38 units lente and 8 units regular insulin daily

When last seen on June 23 1964 she weighed 51.2 kg and her height was 149.3 cm. She is doing very well on 43 units lente and 6 units regular insulin.

Case 2

C S., an 11 year-old white female was admitted to The Children Memorial Hospital for the first time on November 7 1962, with a nine-day history of vague abdominal pains and fatigue For 48 hours prior to her admission to the hospital, she exhibited progressive lethargy anorexia polydipsia, and respiratory difficulty There was no history of vomiting diarrhea or infectious disease exposure She had been seen by a physician about 36 hours prior to admission. At that time a diagnosis of "growing pains" was made, and phenobarbital and vitamin B_{12} were prescribed. There was no family history of diabetes.

Physical examination revealed an acutely ill, dehydrated, comatose girl whose temperature was 36.5°C, pulse 160/min, respirations 40/min, weight 23.5 kg and blood pressure 110/70. All mucous membranes were very dry and the pharynx was moderately injected, but no source of infection was found. The neck was supple lungs clear sinus tachycardia was present and the deep tendon reflexes could not be elicited. The abdomen was scaphoid with very hypoactive peristaltic activity; there was no rigidity tenderness or organomegaly Initial urinalysis showed 4 glycosuria and strong acetone Her biochemical determinations were: CO_2 3 0 mEq/L, sodium 130 mEq/L, chlorid 93 mEq/L, BUN _3 mg%, glucose 540 mg%, and amylase 749 units. The white blood count was 19,200 and the hematocrit 50%. Initial diagnosis was diabetes mellitus and acute pancreatitis. Table summarizes the pertinent chemical findings.




Fig. 1 Fig. 2

Fig. 1 Edematous mucosa of duodenal loop with "picket fence" appearance

Fig. 2 Normal post-pancreatitis duodenal mucosal pattern.

Our diabetic regime was instituted, and she received intramuscular penicillin for four days. On November 8 six hour diabetic management was started, and she was given an antispasmodic and oral tetracycline 250 mg every six hours. A 2200 calorie diabetic diet was started on November 10; at the same time 15 units lente and 4 units regular insulin were administered daily before breakfast.

Her hospital course was one of progressive improvement. A mumps antibody titer drawn on November 14 was negative. Blood, urine and throat cultures were negative, as was an intermediate strength PPD skin test. Chest and gallbladder x-rays were normal. An upper gastrointestinal series on November 8, 1962, was reported as follows: "The stomach appeared normal, the duodenal bulb and loop filled, and it was immediately evident that the mucosal fold of the duodenal loop was excessively wide presumably due to edema. There is hesitation of the passage of the barium beyond the third portion of the duodenum. Spot films demonstrate coarse folds of the duodenum with sharp peripheral ends giving somewhat 'picket fence' appearance compatible with acute pancreatitis" (Fig. 1). A repeat upper gastrointestinal series on November 11, 196_, revealed a normal pattern of the duodenum (Fig. 2).

A follow-up query revealed that the patient is active and doing well in school. In January 1963 she was 139.1 cm tall and weighed 32.2 kg. Her daily insulin dosage was 27 units lente and 19 units regular insulin administered each morning.

In November 1963 she was 142.8 cm tall and 40.1 kg in weight. Her daily insulin dosage was 27 units lente and 22 units regular insulin. She has not begun to menstruate.

Discussion

The clinical course of acute pancreatitis in children is fairly consistent and typical, yet it is a diagnosis that is rarely made, possibly because it is not considered more seriously in the differential diagnosis of the child with an acute abdomen.

In acute pancreatitis, findings consist of sudden onset of abdominal pain in the epigastrium or upper abdomen. The pain

is usually steady and unremitting but it can be vague and intermittent as in the aforementioned Case 2 The pain and clinical picture usually are sufficient to be considered an "acute abdomen There is abdominal pain with tenderness, rigidity and rebound tenderness (often unreliable in children) although the tenderness and rigidity are often less pronounced than one might expect from the severity of the pain. Colicky pain is uncommon. Nausea and vomiting are usually present Abdominal distention is common. Peristaltic activity is usually markedly reduced or often absent since a secondary ileus is frequently found [15] Pleural effusion, Cullen's sign (bluish discoloration of the skin about the umbilicus) or Turner's sign (local discoloration of the skin of the flank) are usually absent Fever is an occasional finding Leucocytosis is usual serum amylase and lipase are usually elevated, the amylase levels rising within 8 to 24 hours of onset of pancreatitis. Serum lipase levels rise somewhat later and may be found in the serum longer than elevated amylase levels The height of serum amylase is not always in direct relation to the severity of the disease About 10% of cases of acute hemorrhagic pancreatitis in adults have normal serum amylase levels [11]. Elevated serum amylase and lipase levels may follow the administration of codeine morphine and demerol [4]

In diabetes mellitus with ketoacidosis, the abdominal pain is not as sudden in onset nor as severe as in acute pancreatitis The pain is also more generalized over the abdomen, generalized tenderness may be present If rigidity is present (rare) it is mild. Abdominal distention is not a feature of diabetic acidosis without pancreatitis Peristaltic activity is usually present but diminished. Headache nausea vomiting and malaise accompany diabetic ketoacidosis. Nausea vomiting and abdominal pain usually subside within four to six hours after the institution of proper intravenous therapy and insulin in the diabetic patient

In diabetic acidosis not due to pancreatitis, there is not a rise in serum amylase or lipase The hyperlipidemia seen in acute pancreatitis is much greater than that seen in uncontrolled diabetes mellitus Case 1 did not have significantly elevated amylase levels, but the lipidemia was so marked that hemoglobin determinations could not be done for several days.

Acute appendicitis and intestinal obstruction are the two most frequently diagnosed entities that can be mimicked by acute pancreatitis. X rays are not always helpful, since ileus often occurs with pancreatitis

There are however two characteristic roentgenographic findings seen in acute pancreatitis. The inflammatory process in the pancreas extends beyond the confines of the organ and affects the normal physiology of the duodenum colon and adjacent peritoneal surfaces. Contrast examination of the duodenum reveals diminished peristalsis dilatation of the second portion of the duodenum coarsening of the duodenal folds, and a delay in the emptying of the duodenum [9]. Excessive accumulation of gas in the ascending and transverse colon with sharp cutoff at the splenic flexure is seen on the supine and upright films of the abdomen. This is probably on the basis of local paralytic ileus [*] Absence of ga

colon does not rule out pancreatitis. The most constant findings are those in the duodenum.

If a laparotomy for acute appendicitis is performed in the presence of unknown pancreatitis, the appendix appears normal and free peritoneal fluid is usually found. This fluid usually has an elevated amylase level.

Walker [14] observed that in diabetic coma the vomiting usually precedes the pain, whereas in precoma with surgical complications the abdominal pain appears before the vomiting.

In differentiating the abdominal pain in acute pancreatitis and in diabetic acidosis, Root [8] mentions three factors which usually indicate pancreatitis: (1) prostration more severe than expected with a systolic blood pressure greater than 100; (2) sudden onset of the abdominal pain with "collapse" in six to ten hours; (3) failure to improve with adequate treatment for diabetes mellitus.

Other conditions which can produce an acute abdomen in children and may confuse the clinician briefly are peptic ulcer, severe gastroenteritis, steroid therapy [3, 6] and (rarely) familial hyperlipemia and its secondary pancreatitis.

Summary

Two children with diabetes mellitus associated with acute pancreatitis have been presented. The significant clinical and radiologic findings of this uncommon association in children and a review of the literature have been presented.

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(H. T.) 6334 N. Broadway
Chicago
Ill., 60626
U.S.A.

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CASE REPORT

Cerebral Gigantism

by BENGT KJELLMAN

From the Department of Paediatrics, University Hospital, Lund, Sweden

In 1964 Sotos et al. [16] described 5 children with symptoms of excessively rapid growth with acromegalic charac teristics and a non-progressive neurologic disease. The skeletal maturity was in all cases increased There were no symptoms of pubertas praecox, thyreotoxicosis lipo-dystrophy eosinophilic hypophyseal tumour Marfan's syndrome or adrenogenital syndrome The excessively rapid growth occurred during the first 4 years thereafter the rate of growth decreased The condition was reported as a new syndrome probably caused by a lesion in th hypothalamic region.

A patient with this symptom complex has in our department been followed for a period of [illegible] years.

Case History

U K. J female born January 15 1960 Birth weight 3400 g length, 53 cm. Parents are below medium height and unrelated. The tw preceding generations showed values below or only slightly bove medium height There are no known genetic diseases among the relatives. A half-brother was healthy and f normal height.

The pregnancy wa normal. The girl was born 3 weeks after the calculated time. Labour was induced by oxytocin drop but otherwise normal. No signs of dysmaturity were noted and the neonatal period was normal. According t the mother the psychomotoric development should have been normal, see below however Weight and height were recorded t the Child Welfare Clinic (see Fig 1). The patient was referred t our department because of gigantism.

On admission at the age of [illegible]6 months the girl could walk but not speak. She was very aggressive wilful, and did not play with her contemporaries. Her hearing seemed normal Her height was 98 cm, which is bove the upper sigma limit for the ge [6, 11], with a sitting height f 55 cm. Her bodyweight was 19.2 kg The overweight in relation to the height—as indicated by the weight figure—seemed t be due t strong habitus with coarse skeleton and very well dev loped musculature (Fig). Abov all, the size of hands and feet was bnormal (Fig 3, 4) length of foot was 1 cm; length of hand 11.5 cm. Her shoe size 28 corresponded t the shoe size for the 5-year old. She had coarse facial features with thick lips and powerful jaws. The skin on hands and feet was rough. She had normal growth f hair and normal genitalia. Her head circumference was 51 cm.

Routine status and neurological investigation proved normal, except that a certain lumsiness could be demonstrated in the fine motorium. Investigation of the field of ision could not be carried out because the patient did not co-operat

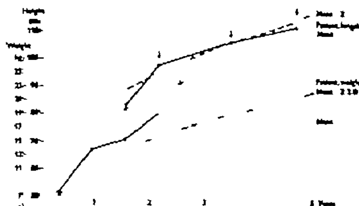


Fig. 1. Weight and length of the patient in relation to mean values and mean + s.D. Arrows indicate the visits to the Children's Hospital.

Laboratory findings

Routine investigations proved blood and urine to be normal. Serum electrophoresis, serum electrolytes (Na, K, Ph, Ca, Cl) serum transaminases and alkalic phosphatases were normal. Serum cholesterol and total lipids in serum were also normal. Nitrogen balance study over a period of four days showed the following nitrogen retention: I 0.4 g N/d, II 4.12 g N/d, III 4.8 g N/d, IV 5.6 g N/d.

Chromosome investigation showed normal karyotype. Cerebrospinal fluid was normal. EEG disclosed no positive deviation from normal. X-ray investigation of cranium and sella turcica was normal. Pneumoencephalography revealed dilatation of the lateral ventricles, the right being somewhat more affected than the left. Septum pellucidum was situated in the median plane. The third and fourth ventricle cisterna basalis and aquaeductus were normal. The pneumoencephalography finding was interpreted as being conditioned by cerebral atrophy. X-ray investigation of the skeleton showed advanced maturity with a hand skeleton development of the upper limit of 4 years according to Schmid & Moll [18].

Peroral glucose loading, protein-bound serum iodide, urine analyses of F.S.H. (follicle stimulating hormone), estrogen and neutral 17-KS and 17-OH-CS showed normal values. SF (sulfation factor)[1] activity in serum showed the value 0.61 units/ml. The upper 2-sigma limit for the age is 0.5 unit/ml [1].

At the age of 3½ years the girl was reinvestigated. She had now developed somewhat. She could speak single words but was

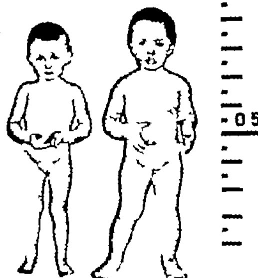


Fig. 2. The patient (right) and normal girl, years and months of age.

[1] Investigation carried out by S. Almqvist, M.D., Stockholm.

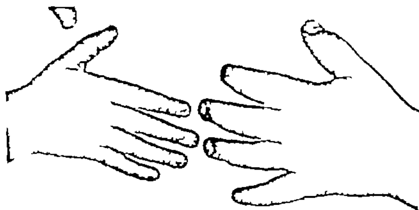


Fig 3. The hand of the patient (right) and normal girl at the age of 2 years and months.

still wilful and aggressive. Her height was 106 cm; span, 107 cm; weight 21.8 kg; length of foot 18 cm; length f hand 13 cm; head circumference 52 cm. The habitus was still very strong. The jaw development in vestigated by odontologist was found to be considerably above the calender age whereas the teeth development was found t be nor mal. Secondary sex characteristics were unexceptional. X-ray investigation of the skeleton showed a hand development cor responding t the ge of 6 years [14] (Fig. 5).



Fig 4. The feet of the pa ien (righ) and normal girl the age of 2 years and 2 months.



Fig. 5. Roentgenogram of the hand at the age of 3 years and 6 months. Bone age 6 years (15).

The hormone analyses carried out earlier were repeated; they proved all normal. Unfortunately, for technical reasons, the SF-activity could not be determined.

Two-dimensional amino acid chromatography of urine revealed normal pattern.[1] EEG now showed a somewhat slow basic rhythm. X-ray showed the cranium and sella turcica to be normal. Electromyelography and determination of conduction velocity of the peripheral nerves showed normal conditions.

At the age of *4 years and 8 months* a third investigation was undertaken. The reason for admission on this latter occasion was nosebleeding. Psychically the patient was now further developed and could speak sentences of three-word length. Her temper had improved somewhat and she could play with other children in the ward. However she was still quite wilful and aggressive. Her height was 11 cm, weight 23.5 kg; length of foot 18.5 cm, length of hand 12.5 cm; head circumference 52 cm. The hand-skeleton development [15] was at the upper limit for age 6 years. EEG now revealed definite abnormality in the form of slow basic activity. X-ray showed the cranium and sella turcica to be normal. The hormone analyses were again repeated and showed normal conditions. Unfortunately, determination of the SF-activity could not be made on this occasion either. CPK (creatinine phosphokinase) in serum was normal. Mucopolysaccharide analysis of urine showed a slight increase of chondroitin sulfate.[2] A complete coagulation study[3] disclosed normal conditions.

Discussion

The patient here reported shows advanced growth in height with marked acromegalic features, her powerful musculature and large hands and feet are particularly noticeable (Fig. 1–4). Furthermore she displays a strongly advanced skeletal maturity (Fig. 5) and signs of non progressive organic cerebral injury as revealed by mental retardation, brain damage behaviour and cerebral atrophy with pathologic EEG.

Endocrine diseases with symptoms of increased growth in height and/or advanced skeletal maturity such as hyperthyreosis, hypercorticoism, and pubertas praecox can be excluded. Lack of hepatosplenomegaly, loss of flesh, and lack of hyperlipaemia and hyperproteinaemia does not agree with the syndrome lipodystrophy and gigantism as described by Berardinelli [5]. The patient has shown no symptoms of neurofibromatosis, in which condition an acromegalic habitus sometimes may appear [8].

[1] Investigation carried out by R. Jagenburg, M.D., Gothenburg.

[2] Carried out by J. Clausen, M.D., Copenhagen.

[3] Carried out by I. M. Nilsson, M.D., Malmö.

Observation of the patient over a period of years and 6 months has not revealed any form of systemic disease

Family information including height for the past two generations renders improbable genetic or constitutional cause of the condition. Furthermore acromegalic features do not usually occur in the constitutional type of increased growth [12].

Our primary diagnosis was gigantism due to eosinophilic hypophysial tumour. In prepubertal children with this disease characteristics of acromegalia have been reported [4 12 18] The skeletal maturity is usually reported to be normal or subnormal [4 1* 18]. In the textbooks normal skeletal maturity is even given as one of the criteria of acromegalic gigantism [17] It is well to not however that most of the well-documented cases of this disease have been investigated at prepuberty or puberty Before this age there are, according to my knowledge so far no cases reported with subnormal skeletal maturity Hurxthal [9] studied a patient with gigantism from the age of 5 years and 8 months up to the age of 16 years. This patient had from the beginning normal bone age but this slowed down later [10] Another two cases with acromegalic gigantism before prepuberty have been published, where obviously the aetiology was eosinophilic hypophysial tumour the skeletal maturity was here markedly increased [13 14]. In the age group below 4 years of age no verified cases of eosinophilic hypophysial tumour seem to have been published, in which an examination of the skeletal maturity has been carried out. In the patient here reported the advanced skeletal maturity per se may thus not be used as a reason for rejecting the diagnosis eosinophilic hypophysial tumour Two facts, however argue strongly against this diagnosis: the normalization later on, of growth rate and furthermore the normal sella turcica X ray during an observation period of ½ years Sotos et al made the same experiences in their patients [16]. The patients reported by Sotos et al., showed great similarities in their facial features It may be noted that the present case also shows great similarities with one of these patients (Case 5)

The patient, here presented has symptoms of brain damage and the encephalography shows enlargement of the lateral ventricles as a sign of cerebral atrophy There are however no signs of increased intracranial pressure The cerebral injury is not progressive during an observation period of ½ years the patient developed considerably psychically as well as motorically In 3 of the 5 patients reported by Sotos et al., encephalography was made which showed different degrees of enlarged ventricle system Signs of increased intracranial pressure could not be demonstrated in these patients either

In the present patient the aetiology and the time of onset of the cerebral injury are not clear According to the data recorded by the Child Welfare Clinic, overweight has existed since the age of 4 months, whereas a normal height reported at the age of 18 months (Fig 1) The height data at this age however are not quite reliable It seems reasonable to presume that the cerebral injury is of prenatal or perinatal origin

During the period of most rapid growth of the patient an SF-act vit increase to above the upper 2-sigma value for the age was established [2] This increase was not

of the size order that usually are found in adult cases of acromegalia [3] However the normal values for children below the age of 4 years lie considerably lower than these for adults The high value here observed suggests that the growth disturbance is at least partly mediated by an increased incretion of growth hormone It is true that the patient lacked certain attributes sometimes seen in classical acromegalia such as abnormal glucose tolerance and tufting of the end phalanges of the fingers The lack of these symptoms does not however exclude a supernormal incretion of growth hormone but might indicate that the incretion was only slightly or moderately increased.

It seems reasonable to presume that in this case the cerebral injury affects at least partly the hypothalamic area causing disturbance of growth rate This case report thus leaves further support to the existence of a special syndrome of gigantism with acromegalic characteristics and a non-progressive neurological disorder If this syndrome has a specific aetiology or is merely an expressi unspecific brain damage is not yet c

Summary

A case of the syndrome cerebra gantism is presented. The patient, a was observed from the age of * years 2 months until the age of 4½ years. had acromegalic characteristics, adva skeleton maturity and showed sign nonprogressive brain damage Com specific aetiology such as eosinop hypophysial tumour pubertas prae thyroid disorders, lipodystrophy and renogenital syndrome could be exclu During the period of most rapid gr an increase of the sulfation factor acti in serum to above the upper *-sigma l for the age was established. As a c previously reported the growth rate normalized later on. It is suggested t in this syndrome the hypothalamic a is affected but whether the syndrome h specific aetiology or is merely an expres of an unspecific brain damage is not cl

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Department of Paediatrics
University Hospital
Lund
Sweden

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Meeting Febr 12 1965

Bengt Eriksson and Claes Thorén Hereditable Disorders of Mesenchymal Tissue with *Congenital Malformations of the Heart*

Some hereditable disorders of mesenchymal tissue with rather well defined syndromes are known to have congenital malformations of the heart In the Holt–Oram syndrome atrial septal defect occurs in 100% of cases, while about 60% of the cases of Ellis–van Creveld syndrome are said to have a single atrium. The Ehlers–Danlos and Marfan s syndromes, however show only a few cases with different congenital malformations of the heart

A mother and two of her four children, both girls, having an earlier not described combination of disorders, were presented. They had a disorder of the connective tissue characterized by changes in the joints, by ...ensibility genu recurvatum and hypermobility of the fingers. The kneejoints showed valgidity which in the elder daughter produced a disabling stagger On the other hand, no abnormality of the skin or bleeding tendency was found. The maternal grandmother had previously had hyperextensible joints.

Typical patent ductus arteriosus of considerable severity as well as dilatation of the systemic arteries were present in all cases. All three were operated on but at different ages. The mother was operated on at the age of 23 years. She had a diffuse aneurysm of the aorta, which 15 years post-operatively appeared to be unaltered. Pulmonary dilatation and pulmonary valvular insufficiency were discovered in the 12-year-old daughter five years after operation. The youngest girl, operated on at the age of 7 months, did not show any cardiovascular disease six years after her operation. The elder daughter had a prolonged AV-conduction time and supra ventricular arrhythmia. From the age of two she has also had epileptic spells of the grand mal type which could be provoked by exercise Vascular abnormalities of the brain may be the cause of the epilepsy

Skin biopsies showed disturbances of the elastic fibres. The ductus of the elder daughter was examined and showed loss of elastic fibres.

The described syndrome is supposed to be a hereditable disorder of the connective tissue with similarities to both Marfan s and Ehlers–Danlos syndromes.

DISCUSSION

Per Zetterqvist The cardiovascular manifestations described—including failure of the ductus arteriosus to close following birth—are probably secondary t the hereditable connective tissue disorder and should here not be considered malformations in the pathogenetic sense. The analogy with Marfan s syndrome is striking. It seems, however inadequate to group this disorder either with Ellis–van Creveld s syndrome which is generally associated with true cardiovascular malformations, or with Holt–Oram s syndrome which is most probably pure malformation syndrome without connective tissue disorder Whether atrial septal defect should be considered necessary manifestation of the latter syndrome is a matter of definition. In addition to a mother and son exhibiting the typical syndrome of

atrial septal defect, heart arrhythmia and hand malformation (described in *Acta Paediatrica* 1963) I have examined a mother and daughter both showing the characteristic arrhythmia. The mother was otherwise normal. The daughter however had the typical hand malformation including bilateral tri-phalangism of the thumb, as well as Fallot anomaly of the heart.

Bengt Persson and Göran Sterky Obesity resistance to Ketosis

In a group of overweight children between 7 and 16 years of age and in 10 normal weight control subjects, two types of tests have been performed: (A) Prolonged fasting (= 24), (B) ketogenic formula diet during one week (= 20).

The following parameters have been determined on venous blood: glucose free fatty acids (FFA), glycerol and ketone bodies.

The obese group shows increased lipolysis in test A compared t the normal, but a lower FFA response. During test B the overweight group shows significantly lower FFA increment compared t the normals. The ketone body response is similar in both groups.

The results could be interpreted as a metabolic adaptation and are discussed against the background of genetic factors

J Winberg et l. Renal Hypertension

A short discussion of different kinds f renal hypertension.

Presentation of one patient with urethral valve bilateral hydronephrosis presenting with diabetes insipidus and hypertensive crisis. After drainage and initial anti hypertensive treatment patient became normotensive then remained normotensive after discontinuation of drugs. Five patients with asymmetric parenchymal reduction, low concentration capacity little or no reduction of glomerular filtration. Urinary findin negative in 4 patients, periodically posit e urine culture in one. High coli anti body titres in four patients. No renal history in two patients, one earlier infection in two, and bacteriuria" in one. They have been judged as patients with silent chronic pyelonephritis. Diagnosis, course and therapy are discussed.

Herbert Enell and Alf W llerström BCG-osteitis

Metastases t the skeleton from a BCG-vaccination in hildren are not particulary rare and must be kept in mind as a possibility in cases of solitary or multiple lesions. The period of time between vaccination and development f symptoms can last some years, the cases reported have been benign. Two such cases are described in detail. Both responded well to antibiotic treatment. Bacteriological examination showed growth of bacteria indistinguishable from the Swedish BCG-strain. Compared to amounts of vaccinations performed, such cases are too few to be used as an argument against the BCG vaccination.

A Aperia, C. G Bergstrand and O Broberger: Studies n Renal Phosphorus Reabsorption in Various P thologic Conditions

The tubular phosphorus reabsorption system has been studied by determining the phosphorus threshold. Characteristic disturbances have been found in hypoparathyroidism, pseudohypoparathyroidism and vitamin D resistant rickets, and in case of subclinical hypoparathyroidism the diagnosis has been verified. The effect of para thyroid hormone (the so-called Ellsworth–Howard test) has been evaluated by the reabsorption index Le phosphorus excretion/100 cc filtrat This has made the test more specific and sensitive which not only increases it diagnostic significance but also helps in determining therapy

Meeting March 13 1965 with Swedish Society for Endocrinology

Symposium: Endocrinological Growth Disturbances

C. G. Bergstrand Linear Growth in Congenital Adrenal Hyperplasia

Linear growth of 14 boys and 14 girl (female pseudohermaphrodites) with congenital adrenal hyperplasia was studied. Eight of the patients showed the salt losing syndrome The following conclusions were reached. Untreated cases may reach an ultimate height which is well within normal limits. Treatment with cortisone started fter the age of 5–6 years, does not as a rule prevent early closure of the epiphyses and (possibly with some exceptions) seems not to produce a definite increase in ultimate height Adequate treatment with cortisone started before the age of 5–6 years, will probably with some exceptions, produce a normal rate of linear growth for several years. As the observation period is still too short it is difficult to predict the final results of this treatment The advanced skeletal development found in some cases in spite of adequat treatment suggests premature closure of the epiphyses and consequently a lower ultimate stature than would be expected from the appearance of the early of the growth curve There seems to be difference in linear growth between salt-ers and patients with a normal lectrolyt regulating mechanism.

Göran Sterky Disorders of Growth in Juvenil Diabetes

The effect of diet insulin, exercise and growth hormone on growth are discussed Data for height and weight are given for diabetics at onset before and after puberty and after 15 years of diabetes. Diabetic boys deviate from normals. They are taller at onset shorter just before puberty show a catch up growth, but are significantly shorter after long duration. Diabetic girls are somewhat older at menarche Dietary restriction must be thoroughly supervised.

Inge Rune Growth Disturbance in Cases of Thyroid Insufficiency

Growth disturbance the commonest symptom of thyroid insufficiency in childhood, was discussed from the diagnostic and prognostic viewpoints on the basis of 18 cases of congenital hypothyroidism. All cases diagnosed before the age of one year or which at later diagnosis had a skeletal age of less than one year were considered to be cases of congenital hypothyroidism.

In 16 of the 18 cases, there was retardation of both length and skeletal age but in all cases, retardation of the latter was more pronounced. Where the illness was of longer duration, body proportions were also affected. In half of the cases not only skeletal retardation but also epiphyseal dysgenesis was present.

The skeletal changes were completely normalised by thyroid treatment within periods varying between 3 to 4 months and 3 to 4 years. S bsequently with adequate substitution therapy growth occurred at normal rate The effect of overdosage was apparent in one case.

Research on apes made hypothyroid at birth has shown that skeletal retardation and reduction of brain weight occur in a parallel manner In these studies, skeletal retardation at the time of treatment as correlated with the IQ after a minimum of 5 years treatment. With the exception of the athyroid cases, who were all mentally retarded even when treatment was initiated early all cases with skeletal retardation of less than one year had a normal IQ while all cases with skeletal retardation of more than one year were mentally retarded.

From observation of these cases, the conclusion can be drawn that it is important to determine the infant rate of growth during the first year of life. At present, this is done after the time when treatment of congenital hypothyroidism should have been initiated.

Meeting April 10 1965

L. E. Carlgren, L. Henricsson G Hansson and P Whalén A Case of Agammaglobuli nemia and F tal B.C.G Dissemination

A boy the only child of healthy parents, was B C.G vaccinated as a newborn without local complication. He was quit w ll up t the age of six months but subsequently was hospitalized for bronchopneumonia and diarrhoea and remained there until his death at nine months of age. Immuno-electrophoresis (Dr C. B. Laurell) disclosed com plete absence of gamma A and gamma M and insignificant amounts of gamma-G (0 09 g/100 ml) There was moderate lymphocytopenia (1000–1500/cu mm) and bone marrow biopsy showed ery few plasma cells. N isohemagglutinins were found. Mantoux 1 100 was negative.

Autopsy showed generalized epithelioid cell granulomatosis, with myriads of acid fast rods, and a few giant cells and areas of caseous necrosis. These changes were parti cularly widespread within bdominal para aortal lymph nodes, spleen and thymus. The latter in addition, was trophic (weigh t 1.5 g) with no Hassll's corpuscles. Beside th specific changes in the lungs there were also signs of unspecific pneumonia Bacteriologic xamination showed that the acid fast bacilli were in all probability of the B C.G strain.

The clinical course, the laboratory findings and thymic pathology in this case strongly speak in favour of a congenital agammaglobulinemia of the lymphopenic (Swiss) type Agammaglobulinemic individuals are generally thought not to be particularly susceptible t tuberculous infection, and successful vaccination has been reported in a few cases. However this and previous report of f tal B.C G dissemination, 8 cases in all the majority of which seem to have had some defect of the gammaglobulin synthesis show that B C G vaccination is not a harmless procedure in patients with congenital agammaglobulinemia, especially in small infants.

I Petersén, O Eeg-Olofsson, I Hagne and U Sellden: EEG in a Material of Selected Healthy Children

Electroencephalographic registrations have been performed in 500 healthy children Photic stimulation, sleep activation and in duction of hyperventilation has been per formed if possible The children had a birthweight not less than 2500 g. They had normal delivery Neither of the f llowing symptoms occurred: disturbances of consciousness, convulsions, nervous abdominal pains or headache enuresis, stuttering, nailbiting, anamnesis of commotio cerebri, convulsive disease in the family and com plications connected with common infections. Somatic and neurologic examination has been carried out

Of the 500 children 245 were boys and 255 girls. Normal EEG was found in 430 children. Some kind of divergent findings in the EEG was recorded in 13% of the children. The majority of those findings were unspecific abnormality with increased amount of theta activity Such an increase f slight unspecific abnormality was found in 49 children and of moderat extent in 9 children. A pronounced unspecific abnormality was not found in any child. In 20 of the 58 children with unspecific bnormality a leftsided predominance was noted and in 4 right sided. (Concerning 436 children with normal EEG a leftsided predominance was noted in 53 and a rightsided in 12.)

Paroxysmal activity occurred as follo s spikes, sharp waves (when awake) – 7 hild ren; spikes, sharp waves (t sleep) – 6 child ren; atypical wave-spike complexes at sleep – 20 children; other paroxysmal act ty – 3 hildren.

28 focal findings were not l, 2 of hich in each of 3 children. The most common localisation was the temporal region 11 cases and the occipital region, 5 cases.

14 and 6 are positive spike (ntals found in 8 of the sleep records.

Rhythmic 2.5–4 per acti ity

temporo-occipital leads was noted from the age of -3 years. Its main percentual representation was in the age of 4–5 years and thereafter it decreased.

Ingrid Hagne Neurophysiological Studies in Enuresis

EEG data in enuretic children, recorded during 195_–64 in the Clinical Neurophysiological Laboratory Sahlgrenska Sjukhuset has been re-examined. The EEG findings have been compared to material covering 385 children in the same age group. In 4 of the enuretics, the motor nerve conduction velocity has been determined in N peroneus and N ulnaris.

The material consists of 122 cases between 4–15 years of age 80 boys and 4_ girls. In 65% enuresis was primary in 30 ,, secondary while 5% could not be classified because of insufficient information. Twenty two children had slight mental retardation. Four had epilepsy two had suffered from febrile convulsions and six from fits of in determinate nature

The EEG was normal in 41% (85 7% in the control material) The positive findings were: non-specific bnormality in 55% (13%), epileptogenic or paroxysmal activity in 7 4 (2.9%) doubtfully epileptogenic paroxysmal in 7 4% (1.3%) and slow sterior rhythm in 20 5% (7%). The difference between the enuretics nd the control material is statistically significant.

The most characteristic EEG finding was a slow rhythmic activity of 2–4 c/sec waves, localized to the posterior temporal, parietal and occip tal regions, most often bilaterally synchronous, sometimes predominating on one side. Such rhythmic activity is seen in a certain percentage of normal children from 3 years of age, reaches a maximum at 5 years and thereafter decreases. In the enuretics, the frequency was between the ages 4–6 years 37.5% (as against 18.7% in the control material), 7–10 years 16 7% (3 1%) 11–15 years 12 9% (2.7%).

The resistance of such activity in older children with enuresis may in some cases be due to delayed neurophysiological maturation. This hythm, however can also ppear in injuries of different kinds.

In our children EEG material, only patients who have undergone heart surgery have had as high as incidence of these rhythms as enuretics. In adult material, a similar rhythm is seen more often in peptic ulcer and convulsions. The rhythm has subcortical character and is supposed to be generated in the diencephalon or the brain stem.

The nerve conduction velocity in enuresis did not differ from the controls.

O Eeg-Olofsson and I Petersén The Diab tic Neuropathy of Childhood

Electrophysiological studies and comparative analysis of clinical data have been performed on 85 diabetic children 15 years old They have been drawn at random from 108 diabeti children controlled at the Children's Hospital, Goteborg Electroencephalography (EEG) was performed in all 85 cases, electromyography (EMG) in 74 cases and determination of the peripheral motor nerve conduction velocity—ulnar and peroneal nerve—in 66 cases.

When analysing the nerve conduction velocity the diabetics and a selected control group of 149 children was divided in two age groups, 3–7 and 8–15 years. A statistically significant reduction of the ulnar and peroneal nerve conduction velocity in the age group 8–15 years was noted in the diabetics as compared to the controls. When values lower than twice the standard deviation below the mean value of the controls was indicated as pathological the ulnar and peroneal nerve conduction velocity was found to be pathological in % respectively 9%. A statistically significant negative correlation was found between nerve conduction velocity and age as well as duration of diabetes.

The EMG analyses showed pathological findings in 5%

For the EEG analyses the diabetics were

divided in the age groups -3 4-6, 7-10 and 11 15 years. Comparison was made t a selected control material of 410 healthy children of the same age groups. Pathological EEG was found in 12% of the controls and in 25% of the diabetics. N relation was noted between pathological EEG findings and age respectively duration of diabetes. 11 of 19 cases with hypoglycemic coma had pathological EEG The alpha-activity was statistically significantly lower in the diabetics compared to the controls. 14 and 6 /sec positive spikes were found in 8% in the controls to 19% in the diabetics which dif ference is statistically significant.

Reports from a Collaborate longitudinal Study of Growth and Development

I grid Klackenberg-Larsson: Mental Development Trends in Children from Three Months t Three Years Old

The children in this study were tested on seven occasions—at 3, 6, 9 12, 18 and 24 months with Brunet-Lezine psycho-motor development test; and at 36 months with Terman-Merrill's intelligence test. The quotients have been normalized so that the mean values are 100. For each child an angle of inclination was worked out for that line which, according t the method of the least squares, is best adapted t a child total quotient values. If the child quotients remained constant throughout the years, this line would be horizontal

The development trend has been related to a number of background variables.

At the ages of 3 and 6 months, the quotient values are higher for children from Swedish social groups II and III than from social group I However between the ages of 18-36 months the opposite is true

The children of mothers with higher education (bove elementary school [*folkskola*] level) tend to have increasing quotient va lues.

A comparison between mothers under 26 years old and mothers 26-45 years old shows that children of the former group ha a decre sing tendency and children of the latter group an increasing tendency

During the period immediately following birth of a sibling the quotient values de crease

A group of severely punished children showed a declining development trend

Development is not significantly affected by the sex of the child or whether its mother is gainfully employed or not.

The variables are often *mutually related* which makes it hard to elucidat the signi ficance of the individual variable

Jan Stensson Corporal Punishment (during the first three years of lif)

The mothers of the children examined were questioned as to the use of corporal punishment By this term is meant any form of punishment intended to cause the child physical pain It is usually no more than a light blow on the fingers or the but tocks.

Eighteen months is the age when both boys and girls are punished most by their parents (both mother and father) The breakdown figures for children punished *at least once a week* are

	Per cent
Boys punished by mother	85
Girls punished by mother	72
Boys punished by father	80
Girls punished by father	26

A cross-section analysis of punishment ad ministered t least once a day at the age of 12 and/or 18 months gave the following result. The most definite relation was that existing between the mother age and the time of conception (conception outside or before marriage as against conception within matri mony) with a higher frequency of daily punishment among the younger group of mothers (under 25 years) and among mot hers who conceived before or outside mat rimony However it should also be taken int account that these groups live in poorer circumstances, with inferior housing conditions, and so on, which increases the possibility of domestic conflict

G Klackenberg: Expectations and Reality concerning Toilet Training

This study is a part of the prospective longitudinal investigation of 212 city children, chosen at random from an antenatal clinic. The author gives a short account of the time when training starts, of the average length of training and of the time when the children achieve urinary and bowel control. The median age of first attempt at training is for girls 8 months and for boys 9 months. The time of steady control of micturition and defecation varies independently of the time of first attempt at training. Consequently early training does not produce earlier results than late training but on an average it gives longer duration of training.

The median ages (in months) of function control are shown in the following table:

	Urinary control		
	Daytime	Night	Bowel control
♀	24	[illegible]	21
♂	[illegible]	[illegible]	[illegible]

The difference between the sexes is probably significant for urinary control ($P < 0.05$) and significant for bowel control (P 0.001). Significant age and sex differences are found concerning the frequency of active resistance and defecation afterwards, boys being more troublesome than girls and the usual age for trouble is 18 months.

The timepoint of reliable control did not appear to be statistically related to social status or to intelligence (within the range of Terman–Merrill quotients between 70–150 at 3 years).

Meeting May 26 1965

Irène Sjögren, J O Bennetter and A Killander: Purpura, Thrombocytopenia, Hyperlipemia and Hepatosplenomegaly in Rubella Embryopathy

Five infants were born during October and November 1963 to mothers who had had rubella during the first to the sixth weeks of pregnancy. Besides three or four of the "classical" symptoms of rubella embryopathy all of them also had purpura, thrombocytopenia, hepatosplenomegaly, a rise in the total serum lipids and carrot red hair. These symptoms have been reported only in a few cases before. All the infants had normal megacaryocytes on bone marrow examination; one had leucocytic inclusion bodies of Döhle type. The purpura and thrombocytopenia disappeared in the first few months of life and the hepatosplenomegaly during the first year.

In other countries live rubella virus has been demonstrated in the urine and in the nasal mucus of new born babies with rubella embryopathy.

Therefore it is reasonable to conclude that the picture of acute perinatal infection in these infants at birth was due to live intracellular virus acquired at the time when the mothers had rubella.

Bengt H Persson and Lars Wranne: Intrauterine Transfusion of the Foetus in Haemolytic Disease

Liley has introduced the intrauterine transfusion of the foetus in haemolytic disease. The following is a report of a successful trial of this technique.

The mother, aged 28, was pregnant for the fourth time. Her first child is alive. The second was born alive but hydropic at 3[illegible] weeks gestation. It died at 40 min of age. The third child was born at 31 weeks gestation, severely hydropic and asphyxiated and died at the age of two days despite several exchange transfusions and continuous artificial respiration. In the 25th week of her fourth pregnancy the indirect Coombs titer was 1/160 and the papain titer 1/320. In the 30th, 31st, 33rd and 34th weeks, specimens of amniotic fluid were analysed according to Liley. The optical densities

at 450 mμ were 0.20; 0.1; 0.17 and 0.16, respectively. This was thought to indicate severe erythroblastosis of the foetus. At 34th weeks gestation, intrauterine transfusion was performed according to the principles of Liley. Fifty millilitres of washed concentrated O Rh-negative erythrocytes were injected into the peritoneal cavity of the foetus. On the fifth day following the transfusion, labour started spontaneously and a boy weighing 1930 g was born. Apgar score was 10. The haemoglobin concentration of the cord blood was 9.5 g % and the bilirubin concentration 5.1 mg%. The Coombs direct test was positive and the blood group A Rh positive. By differential agglutination, 34% of the erythrocytes were found to be O Rh-negative donor cells. Fifty-six per cent of the cord blood haemoglobin was alkali resistant. There was no oedema. The boy grunted during the first 16 hours of life and was given oxygen to relieve cyanosis. However, there were no inspiratory retractions. The boy's scrotum, in special its left side, was distended by a haematoma. This might have been due to the passage of blood through an open tunica vaginalis. The first exchange transfusion was started 30 min after birth and three additional exchanges were performed during the first 48 hours. A short cardiac arrest during the fourth exchange transfusion was successfully treated by external cardiac compression. A fifth exchange was necessary on the fourth day of life to prevent excessive bilirubin levels. After that, the course was uneventful and the boy was discharged at the age of 8 weeks weighing 2850 g. The transfused quantity of haemoglobin was about 10 g. The amount found in the infant circulation, however, was only some 6 g. The fate of the remaining 4 g of haemoglobin is not accurately known.

A. G. Reutersköld and Ingrid Sjögren: Spitz-Holter Shunt Operations on Hydrocephalus Material. Preliminary report

At the Department of Pediatrics and Pediatric Surgery, University Hospital, Uppsala, 61 children (38 boys and 23 girls) with expansive infantile hydrocephalus, born in 1960 and thereafter, were treated during the period between 1.1.1961 and 30.6.1964. In every case the diagnosis was verified by echo-encephalography and pneumo-encephalography and/or ventriculography and in doubtful cases the patency of the aqueduct was tested by I[131] Diodrast disappearance studies.

Patients who did not show a rapidly expanding hydrocephalus were not operated on because of the relatively good prognosis of spontaneously arrested hydrocephalus, and the unknown risks of the operation when started. No child was disqualified from operation because of malformations in the brain itself.

The material and the mortality rate in the operated and non-operated groups are presented below:

Type of hydrocephalus	Operated		Non-operated		Total
	Alive	Dead	Alive	Dead	
Simple	18		8	1	[illegible]
With spina bifida cystica	4	4	1	4	13
With other malformations of the C.N.S.	10	4	4	0	18
Due to Plexus chorioideus papilloma	0	1	0	0	1
Total	32	11	13	6	61

Forty three patients were operated on. Eleven (25%) died. Seventeen (40%) were operated on before the age of 2 months, 33 (75%) before they were six months old. When the shunts seemed not to function, the children were re-operated. This happened in 20 of the 32 cases. Infection of the shunt *mostly caused by staphylococci albi* was the chief cause of death (6 of 11 deaths). The clinical picture of infection was intermittent fever splenomegaly anemia and a positive blood culture after pumping the valve. The infection lodges in the valve: it is almost impossible to get rid of it without removing the shunt and inserting a new one after intensive treatment. The results of operation in children with "simple" hydrocephalus was comparatively good. The mortality rate of the children with complicating malformations is higher after operation, but the operation saves the survivors from developing a very large head and they will be much easier to take care of ("nursery indication").

Our material is a selected material. The children most seriously affected with hydrocephalus were sent to us and the gravest cases were operated on. More than 50% had hydrocephalus in combination with other malformations. A comparison of this material with an earlier study of the natural *prognosis of untreated hydrocephalus*, in which the incidence of hydrocephalus complicated by other malformations was only 25%, showed a survival rate of 75% for the operated material, but only 50% for the untreated material. The mortality rate will, however probably increase for the operated material, as the observation period has been too short.

Today we are more inclined to operate especially on cases with simple hydrocephalus, where the grey cells are not primarily damaged and where a successful operation may produce quite healthy children, psychically as well as physically.

R. Lagercrantz, Stockholm

NEW BOOKS RECEIVED

Books received by *Acta Paediatrica Scandinavica* are acknowledged under this heading. Selected books will be reviewed in subsequent issues space permitting

J ku Gontrea. Die richtige Ernährung der schwangeren und der stillenden Frau und ihre Bedeutung für die Gesundheit von Mutter und Kind Gustav Fischer Verlag, Jena, 1965 386 pages, illustrated. Price DM 61

Pal Gegesi Kiss Krankheit und Heilung Akadémia Kiadó, Budapest, 1965. 231 pages.

Ulrich Keuth Das Membransyndrom der Früh und Neugeborenen Springer Verlag, Heidelberg–New York, 1965. 153 pages, illustrated. Price DM 36.

Milton Markowitz and A Gayler Kuttner Rheumatic fever Diagnosis management and prevention Vol. 11 in the series Major problems in Clinical Pediatrics, Alexander J Schaffer Consulting Editor W B Saunders Company Ltd, London, 1965 42 pages, illustrated. Price £2 12s. 6d

G De Toni. Auxologia Volume Primo. Auxologia prenatale Edizioni Minerva Medica Torino, 1965 830 pages, illustrated. Price £16 000

J Bergès and L. Lézine The imitation of gestures Translated by Arthur H Parmelee Clinics in development l medicine The Spastics Society Medical Education and Information Unit in association with William Heinemann (Medical) Books Ltd, London, 1965 116 pages, illustrated. Price 21s.

L. Meyler and H M Peck (Ed) Second symposium on drug-induced diseases. Excerpta Medica Foundation, 1965. 163 pages, illustrated. Price $ 12.60.

BOOK REVIEW

Jak G Howells (ed.) Modern Perspectives in Child psychiatry

Oliver & Boyd Ltd Edinburgh and London, 1965. Price £ 5 5

This book is the first volume in a series Modern Perspectives which will be published within the psychiatric field. The purpose is not to cover the whole field but to elucidate those parts where the advancement has been especially rapid and substantial.

This child psychiatric volume has two principal parts. The first one general part occupies almost half of the book and is written by number of well known psychologists. As an introduction the present status of the child psychiatric field of research is described in gross traits amongst others with etiological and genetic considerations. Various aspects on clinical psychology follow and this part closes with chapter on the special psychology that is met among in-

ividualistic overintelligent children and handicapped children.

The other special part of the volume deals almost exclusively with the psychiatry of adolescence. Psychosomatic aspects, speech disturbances, accident proneness, juvenile delinquency, suicides and suicidal attempts are given special attention. One chapter deals with neuro-psychiatry and another with psychoses. In some minor part the organisation and the work at the child psychiatric department, the treatment (elucidated by one case report) and the methods of examination are discussed.

As a whole the book is uneven. Even if certain chapters give wide views and are very informative and this is especially true about the general part of the book, other chapters consist of recapitulations of wh has been written previously. It is, howev a great merit that such important fields speech disorders, accident proneness, suicid attempts and juvenile delinquency are di cussed in detail. In an eventual new editi the name Kraepelin, one of the foremost i psychiatry, should be spelled correctly.

As a conclusion it may be said that th book is well worth reading and to be recom mended above all due to the high standar of the general part. The clinical part ma probably be of interest to the physician wh has a general medical interest but is ha very little to offer to the specialist.

Ingvar Nylander, Stockholm

ANNOUNCEMENT

The division of Continuing Education, The University of Texas Graduate School of Biomedical Sciences at Houston, in collaboration with The University of Texas M. D. Anderson Hospital and Tumor Institute is presenting a two-week course in Cancer Chemotherapy May 9 to 21 1966. The chemistry, pharmacology and clinical applications of the antimetabolites, alkaloids, alkylating agents, antibiotic hormones and a miscellaneous group of newer drugs will be reviewed as well as the approaches in current clinical drug assessment and the management of the cancer patient. The course will be a comprehensive in-depth review of the subject.

For further information write to: Division of Continuing Education, The University of Texas Graduate School of Biomedical Sciences at Houston, 107 Jesse Jones Library Building, Texas Medical Center, Houston, Texas 77025 U.S.A.

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